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Original Research Article

Preventive effects of geraniol in schizophrenia-like symptoms in mice models of psychosis

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ABSTRACT

Background: The pathogenesis of schizophrenia has been linked to N-methyl-D-aspartate receptor (NMDAr) inhibition and DAr hyperfunction. Geraniol is a naturally occurring acyclic monoterpene with diverse pharmacological applications. We aimed to assess the effect of geraniol on schizophrenia-like symptoms, *vis a vis* its modulatory actions on neurochemicals in mice models of psychosis.
Methods: In acute studies, male Swiss mice (n=5/group) were intraperitoneally treated with geraniol (25, 50 and 100 mg/kg), risperidone (0.5 mg/kg) or vehicle (10 ml/kg) prior to ketamine (KET) (10 mg/kg)-induced stereotypy and hyperlocomotion. In the chronic studies, mice (n=7/group) were exposed to 14 days interventions (geraniol or risperidone) following a preventive treatment with KET (20 mg/kg) from days 7-14 consecutively. The effects of treatments (e.g., geraniol or risperidone) alone and on KET-induced schizophrenia-like symptoms were investigated on the last day, 24 hours after treatments. Following that, neurochemical and neurotrophic alterations in the brain (striatum, prefrontal cortex, and hippocampus) tissues were investigated.
Results: Intoxication with KET was associated with schizophrenia-like symptoms as evidenced by stereotypy behavior and hyperlocomotion. KET further induced hyperlocomotion, behavioral despair, and cognitive impairment in the chronic studies. It altered the levels of dopamine, 5-hydroxytrypamine, glutamic acid decarboxylase (GAD), acetylcholinesterase (AChE), and brain-derived neurotrophic factor (BDNF) in brain tissues. However, GER (50 and 100 mg/kg) administration significantly prevented the brain's insults caused by KET.
Conclusions: Altogether, the findings support geraniol's neuroprotective activity while also adding to the body of knowledge that geraniol inhibits schizophrenia-like symptoms via modulation of neurochemical and neurotrophic pathways.

Keywords: Brain derived-neurotrophic factor, Geraniol, Ketamine, Neurotransmitters, Psychosis

INTRODUCTION

More than a disease, schizophrenia (SCZ) is a neuropsychiatric syndrome marked by three major categories of symptoms: positive (e.g., hallucination and delusion), negative (e.g., lack of interest and asociality), and cognitive (e.g., loss of learning and memory) features.[1] It affects approximately 1–1.5% of the global population, with a rising prevalence among young adults, and a male-to-female ratio of 1.4 to 1.0.[2,3]

Although the etiology of this condition is not fully known but the knowledge of the interaction between genetic and environmental factors has led to several hypotheses of the illness, with neurochemical dysregulation being at the forefront and it is one of the most well-explored hypotheses in the search for new primary or adjuvant

antipsychotics with better therapeutic benefits.[4-6] Although hyperdopaminergic neurotransmission in the mesolimbic system has been the most challenging pathophysiology of the disease, numerous neurochemical dysregulations such as perturbations in acetylcholinergic, serotonergic, glutamatergic, and gamma amino butyric acid (GABA), notably, clinical findings have shown depleted levels of GABA evidenced by decreased glutamic acid decarboxylase (GAD) mRNAs expression in the brains of patients with schizophrenia and animals exposed to preclinical models of schizophrenia.[4,6-9] Specifically, treatment of animals with dopaminergic agonists such as apomorphine, amphetamine and ketamine (KET) have been reported to characterize psychotic phenotype which are linked to neurochemical dysregulations.[4,6,7]

Notably, neuroscientists are getting more interested in natural medicine for the treatment of psychotic conditions such as SCZ, bipolar disorder, depression, epilepsy and Alzheimer's disease, and a number of phytoconstituents or compounds from natural products, including geraniol, have previously been associated to have therapeutic benefits. Geraniol (3,7-dimethylocta-trans-2,6-dien-1-ol) is a natural acyclic monoterpene found in a wide range of herb oils (e.g., rose, nutmeg, ginger, palmosoral, cilantro, orange, lavender, and lemongrass).[10,11] Its isolates from plants (such as *Monarda fistulosa*, *Cinnamomum tenuipilum*, *Dracocephalum moldavica*, *Valeriana officinalis*, *Rosa damascene*, and others) have been shown to exhibit anti-microbial, anticancer, anti-ulcer, antioxidant, anti-inflammatory, and neuroprotective activities *in-vivo* and *in-vitro* investigations.[12-18] Interestingly, geraniol supplementation have been reported to mitigate motor disturbance seen in rotenone- and 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced Parkinson's disease, as well as prevent cognitive impairment and other neurotoxicity phenotypes induced by D-galactose, zinc oxide nanoparticles, lipopolysaccharide, acrylamide, and streptozotocin brain insults by modulating various signaling pathways.[18-22] Previous studies have also reported that geraniol produced CNS depressant-like effect and anticonvulsant-like activity in pentylenetetrazole-induced convulsion via augmentation of the GABA system.[23] Nevertheless, in the context of SCZ, which is a neuropsychiatric disease excessively associated with behavioral hyperactivity, there is no data on the effect of geraniol on schizophrenia-like phenotypes, as well as its plausible mode of action and interaction in animal model of psychosis. Thus, this study hypothesizes that preventive administration of geraniol would avoid SCZ-like behavior, which may be linked to normalization of homeostatic dysregulation of neurotransmitters and neurotrophic factors.

METHODS

Study type

The study was an original research work.

Study place

The study was conducted at the neuro-pharmacology laboratory of the Department of Pharmacology, University of Port Harcourt, Rivers State, Nigeria.

Study period

The study was conducted from October 2019 to September 2022.

Ethical consideration

The experimental protocol was reviewed and approved by the University of Port Harcourt Ethical Committee with ethical number (UPH/CEREMAD/REC/MM83/037).

Experimental animals

Male Swiss mice (20-25 g) were obtained from the laboratory animal house of the University of Port Harcourt in Nigeria. Animals were housed in an air temperature-controlled environment (23±2 ^{0}C) with a 12-hour light: 12-hour dark cycle, relative humidity 40-70%, and access to food and water *ad libitum*. All procedures in this study followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication no. 85-23, revised 1985) and the University's animal ethical law.

Drugs and chemicals

Ketamine hydrochloride (Rotex Medica, Germany), geraniol, risperidone adrenaline, Ellman reagent, sodium chloride (NaOH), neurotransmitter (dopamine, glutamate, and serotonin), enzyme-linked immunosorbent assay (ELISA) kits (Abnova, Germany), were all used in the study.

Drug preparations and treatments

Normal saline was used to dissolve geraniol (GER), risperidone (RIS), as well as to dilute KET. Mice were given intraperitoneal (i.p.) injection of vehicle (VEH) (normal saline, 10 ml/kg), GER (25, 50 and 100 mg/kg), RIS (0.5 mg/kg), KET (20 mg/kg). Doses of geraniol (25, 50 and 100 mg/kg) used in this study were chosen based on the findings from preliminary investigations.[24] The doses of RIS used in this study were adopted from previous studies.[4] RIS, which served as positive control was used.

Experimental design

Effect of geraniol on ketamine-induced stereotypy

Ketamine-induced stereotypy were employed to screen for the antipsychotic effect of geraniol according to the method described.[6] The mice were randomly divided into five (5) treatment groups (n=5/group). The group one was pretreated with vehicle [10 ml/kg, per oral (i.p.)] (serving as negative control) while group 2–4 was pretreated with

geraniol (25, 50, and 100 mg/kg, i.p.), while group 5 was pretreated with RIS (0.5 mg/kg, i.p.) as positive control. Sixty minutes thereafter, each animal in groups 2 to 5 received intraperitoneal injection of sub-anaesthetic dose of KET (10 mg/kg), and mouse was placed immediately at the center of a transparent open field chamber (35×30×23 cm) to assess the duration number of line crossings for 5 min using a stopwatch. Thereafter, stereotypy behavior was observed for 2 min at 5, 10, 20, 30, 40, and 50 min based on earlier study protocol.[6]

Preventive effect of geraniol on ketamine-induced schizophrenia-like behaviors, and neurochemical alterations in mice

The preventive effect of geraniol on KET-induced SCZ-like behaviors, neurochemical, and neurotrophic, alterations were evaluated as previously described in three different divided studies.[4,25] In study one (drug alone), mice were grouped into 5 experimental groups (n=7/group). Group 1 was received vehicle (10 ml/kg, i.p.) treatment, groups 2, 3 and 4 were administered geraniol (25, 50 and 100 mg/kg, i.p.), groups 5 received RIS (0.5 mg/kg, i.p.) for 14 days, respectively. In the preventive protocol, mice were randomized into 6 experimental groups (n=7/group). Group 1, which served as normal control was given vehicle (10 ml/kg, i.p.), group 2 received vehicle (10 ml/kg, i.p.) and served as negative control, groups 3-5 received geraniol (25, 50 and 100 mg/kg, i.p.), while groups 6 received RIS (0.5 mg/kg, i.p.), serving as positive control, for 14 days. Between the 8th and 14th day of treatment, the animals in groups 2-6 additionally received a daily dose of KET (20 mg/kg, i.p.) or vehicle 30 min after geraniol, RIS administrations respectively.

Behavioral tests

The effect of geraniol was evaluated on KET-enhanced immobility in forced swim test paradigm (representing negative symptoms), was assessed as described with minor modification.[6,26] Antipsychotic effect on the negative symptoms was based on reduction in immobility time as previously described.[6] The effect of geraniol on ketamine-induced non-spatial working memory impairments was also assessed based on discrimination index (DI) using novel object recognition test (NORT), as previously described.[27]

Preparation of brain tissues for spectrophotometric and enzyme-linked immunosorbent neurochemical assays

Immediately after the behavioural tests, mice (n=5) in the respective groups were sacrificed under ether anesthesia and the brains were rapidly removed. The whole brains were weighed and dissected into specific brain regions (striatum, prefrontal cortex, and hippocampus) on a cold ice tray at 4^{o}C. Thereafter, the striatum, prefrontal cortex, and hippocampus tissues were singly homogenized with 5 ml of 10% w/v phosphate buffer (0.1 M, PH 7.4) respectively. Each brain tissue homogenates were centrifuged at 10,000 g for 10 min at 4^{o}C, the pellet was discarded and the supernatants were immediately separated into various portions for the different biochemical assays.

Determination of brain neurochemicals

Regional brain DA and 5-HT levels of the striatum, prefrontal cortex, and hippocampus were estimated in mouse brain using ELISA kits (Abnova, Germany) according to the manufacturer's instructions. All reagents, standard solutions and samples were brought to room temperature before use. In the assay of GAD levels. Its assay was performed as previously described by acetylcholinesterase (AChE) enzyme activity, a marker for cholinergic neurotransmission, was measured in the striatum, prefrontal cortex and hippocampus by the Ellman's assay as described.[28,29]

Determination of brain derived-neurotrophic factor levels

Regional brain derived-neurotrophic factor (BDNF) levels of the striatum, prefrontal cortex and hippocampus were determined in mouse brain using ELISA kits (Abnova, Germany) according to the manufacturer's instructions. All reagents, standard solutions and samples were brought to room temperature before use.

Statistical analysis

Values were expressed as means±SEM using GraphPad Prism 5 software (San Diego, CA, USA). One-way analysis of variance (ANOVA) was used to analyze the result of behavioral tests and neurochemicals. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of geraniol on KET-induced stereotypy in mice

As shown in Table 1, KET (10 mg/kg, i.p.) significantly ($p<0.05$ and $p<0.001$) increased stereotypic behavioral activity compared to vehicle control. Pretreatment with geraniol (25 mg/kg, i.p.) did not elicit significant protective effect against KET-induced stereotyped behaviors at 5-50 min time intervals compared to KET (10 mg/kg)-treated group. However, pretreatment with higher doses of geraniol (50 mg/kg, i.p.) produced a significant ($p<0.05$ and $p<0.001$) inhibition of stereotyped behaviors at 10-50 min time points relative to KET control group. Geraniol (100 mg/kg, i.p.) produced a profound significant ($p<0.01$ and $p<0.001$) reduction in stereotypy score at 5, 10-, 20-, 40- and 50-min interval when compared with KET treated group alone. Furthermore, RIS (0.5 mg/kg, i.p.) significantly ($p<0.001$) inhibited stereotyped behaviors from 5–50 min time intervals in comparison with KET control respectively (Table 2).

Effects of geraniol on naive and ketamine-enhanced immobility in forced swim test in the preventive treatment

Figure 1a shows the effect of geraniol on behavioral despair, based on duration of immobility using forced swim test. Administration of geraniol (25, 50 and 100 mg/kg) and RIS (0.5 mg/kg, i.p.) did not produce any significant [F (4, 30) =3.262, p=0.0246] changes in the duration of immobility in the FST relative to saline (10 mL/kg, i.p.).

As shown in Figure 1b, intraperitoneal injection of KET (20 mg/kg) significantly ($p<0.05$) increased the duration of immobility in the FST in the preventive treatment protocol in comparison with saline-treated group, which suggests behavioral despair indicative of negative symptom, as shown in Figure 1b. However, preventive study with geraniol (100 mg/kg, i.p.) elicited a significant ($p<0.001$) [F (5,36) =6.505, p=0.0002] decrease in immobility time when compared with KET-treated group (Figure 1b). Treatment with the atypical antipsychotic standard drug, RIS (0.5 mg/kg, i.p.) significantly ($p<0.01$) attenuated KET-induced enhanced immobility time in the FST when compared with KET treatment alone.

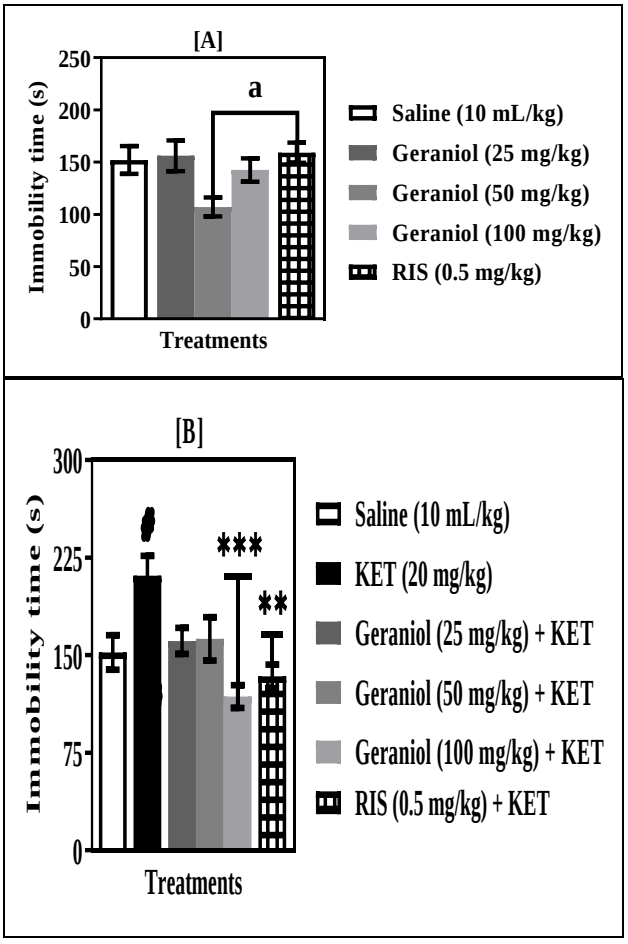

Figure 1: Effects of geraniol on naïve (a) and ketamine-enhanced immobility in forced swim test in the preventive (b) treatment.

Bars represent the mean±S.E.M of 7 animals/group; [a]$p<0.05$ compared to RIS group, #$p<0.05$ compared to saline group, and *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to KET group (one-way ANOVA followed by Bonferroni post-hoc test); KET=ketamine, RIS=risperidone.

Geraniol attenuates ketamine-induced dopamine concentrations in the striatum, prefrontal cortex and hippocampus in the preventive treatment

The effects of geraniol on KET-induced changes in dopamine concentrations in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive treatment with ketamine is shown in Figure 2. In the preventive treatment, KET (20 mg/kg, i.p.) significantly increased dopamine concentration in the striatum ($p<0.001$), prefrontal cortex ($p<0.001$) and hippocampus ($p<0.05$) (Figure 2). Geraniol (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) treatments significantly ($p<0.001$) reduced the increased dopamine concentration in the striatum and hippocampus ($p<0.001$) when compared with KET-treated mice (Figure 2). Also, geraniol (100 mg/kg, i.p.) ($p<0.01$) and RIS (0.5 mg/kg, i.p.) ($p<0.001$) prevented KET-induced increased dopamine release in the prefrontal cortex relative to KET group.

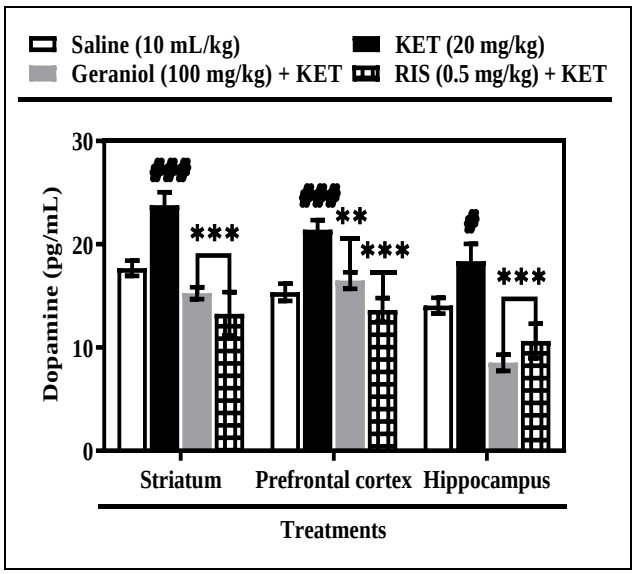

Figure 2: Preventive effect of geraniol on dopamine concentrations in the striatum, prefrontal cortex and hippocampus of mice treated with ketamine.

Bars represent the mean±S.E.M of 7 animals/group; #$p<0.05$, ###$p<0.001$, compared to saline group and *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to KET group (two-way ANOVA followed by Bonferroni post hoc test); KET=ketamine, RIS=risperidone.

Geraniol reduces ketamine-induced alteration in serotonin (5-HT) concentrations in the striatum, prefrontal cortex and hippocampus in the preventive treatment

The effects of geraniol on KET-induced changes in 5-HT concentrations in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive treatment with ketamine is shown in Figure 3. In the preventive

treatment, KET (20 mg/kg, i.p.) significantly increased 5-HT concentration in the striatum ($p<0.001$) but not in the prefrontal cortex and hippocampus (Figure 3). However, geraniol (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p<0.001$) prevented the increase in 5-HT concentration caused by KET in the striatum when compared with KET treatment (Figure 3).

Table 1: Effect of geraniol on ketamine-induced stereotypy in mice.

Treatments	Stereotypy score					
	5 min	10 min	20 min	30 min	40 min	50 min
SAL (10 mL/kg)	1.67±0.27	1.41±0.83	1.19±0.73	1.38±0.13	1.45±0.36	1.10±0.07
KET (10 mg/kg)	4.17±0.37$^{\#}$	4.93±1.03$^{\#}$	5.67±0.64#	5.13±0.83$^{\#}$	6.03±1.63#	4.03±0.13#
Geraniol (25 mg/kg) + KET	4.60±0.84	4.73±0.73	4.73±0.32	4.07±0.36	5.25±0.63	3.92±0.26
Geraniol (50 mg/kg) + KET	3.93±0.19	3.01±0.86^{a}	2.65±0.67^{b}	2.04±0.28^{c}	2.16±0.23^{c}	1.19±0.02^{c}
Geraniol (100 mg/kg) + KET	3.89±0.67	1.98±0.23^{c}	1.66±0.43^{c}	0.98±0.01^{c}	0.75±0.01^{c}	0.00±0.00^{c}
RIS (0.5 mg/kg) + KET	1.67±0.20^{c}	0.75±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}

Value represents the mean±S.E.M of 5 animals/group. Data were analyzed by two-way ANOVA, followed by Bonferroni post-hoc test: #p<0.001 compared to saline group; a = p<0.05, b = p<0.01, c = p<0.001 compared to KET group. SAL=saline, KET=ketamine, RIS=risperidone.

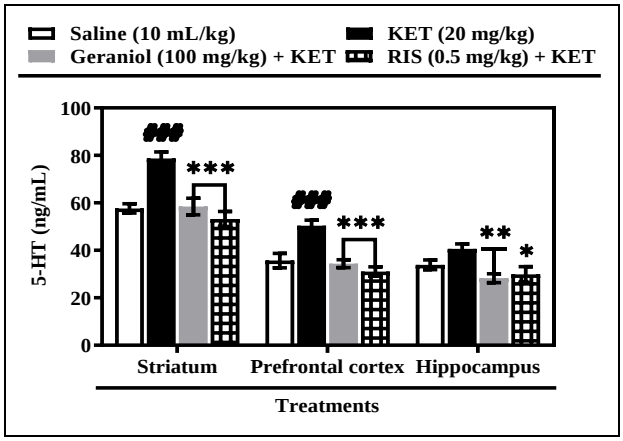

Figure 3: Effect of geraniol on 5-hydroxytrptamine (5-HT) concentrations in the striatum, prefrontal cortex and hippocampus in the preventive treatment with ketamine.

Bars represent the mean±S.E.M of 7 animals/group; ###p<0.001 compared to saline group; *p<0.05, **p<0.01, ***p<0.001 compared to KET group (two-way ANOVA followed by Bonferroni post hoc test); KET=ketamine, RIS=risperidone.

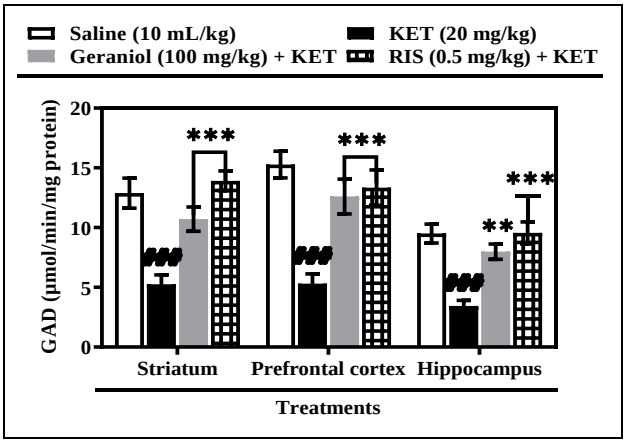

Figure 4: Geraniol up-regulates GAD concentrations in the striatum, prefrontal cortex and hippocampus in the preventive treatment with ketamine.

Bars represent the mean±S.E.M of 7 animals/group; #p<0.05, ###p<0.001 compared to saline group; *p<0.05, **p<0.01, ***p<0.001 compared to KET group (two-way ANOVA followed by Bonferroni post hoc test); KET=ketamine, RIS=risperidone.

Geraniol up-regulates GAD concentrations in the striatum, prefrontal cortex and hippocampus in mice treated with ketamine

The effects of geraniol on KET-induced changes on GAD concentration in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive treatment with ketamine are shown in Figure 4. Here, KET (20 mg/kg) significantly reduced GAD concentration in the striatum ($p<0.05$), prefrontal cortex ($p<0.01$) and hippocampus ($p<0.01$) (Figure 4). In the striatum, prefrontal cortex and hippocampus, geraniol (100 mg/kg, i.p.) and RIS (0.5 mg/kg, i.p.) significantly ($p<0.01$, $p<0.001$) increased GAD concentrations when compared with KET group (Figure 4).

Preventive effect of geraniol on ketamine-induced changes in brain derived neurotrophic factor (BDNF) concentrations in the striatum, prefrontal cortex and hippocampus of mice brains

The effects of geraniol on KET-induced changes on BDNF concentration in the striatum, prefrontal cortex and hippocampus of mice brains in the preventive treatment with ketamine are shown in Figure 6. Ketamine (20 mg/kg) intraperitoneal injection significantly reduced BDNF concentration in the prefrontal cortex ($p<0.01$) and hippocampus ($p<0.01$) but not in the striatum in the preventive treatment (Figure 6). Geraniol (100 mg/kg, i.p.) ($p<0.05$) significantly increased BDNF concentrations in the prefrontal cortex and hippocampus when compared with KET group. Although risperidone (0.5 mg/kg, i.p.) did not produce any significant increase in BDNF level in the prefrontal cortex, it increased BDNF level in the hippocampus when compared with KET group (Figure 6).

Effects of geraniol on AChE activity in the striatum, prefrontal cortex and hippocampus of naïve and ketamine-treated mice

Intraperitoneal injection of ketamine (20 mg/kg, i.p.) significantly increased AChE activity in the prefrontal cortex and hippocampus ($p<0.05$) but not in the striatum (Figure 5). However, geraniol (25, 50 and 100 mg/kg, i.p.) ($p<0.001$, $p<0.01$) and RIS (0.5 mg/kg, i.p.) ($p<0.001$) significantly reduced the AChE levels in the prefrontal cortex and hippocampus when compared with KET treated mice. In the striatum, only geraniol (100 mg/kg, i.p.) ($p<0.01$) reduced AChE activity relative to KET control (Figure 5).

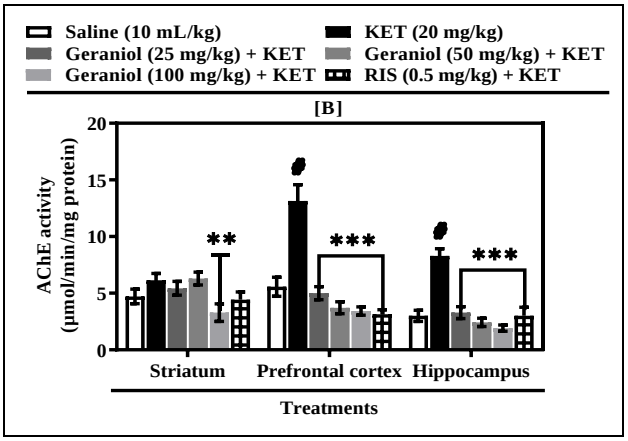

Figure 5: Effects of geraniol on preventive treatment with ketamine on acetylcholinesterase (AChE) activity in the striatum, prefrontal cortex and hippocampus of mice brains.

Bars represent the mean±S.E.M of 7 animals/group; #$p<0.05$, ##$p<0.01$, ###$p<0.001$ compared to saline group; *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to KET group (two-way ANOVA followed by Bonferroni post hoc test); KET=ketamine, RIS=risperidone.

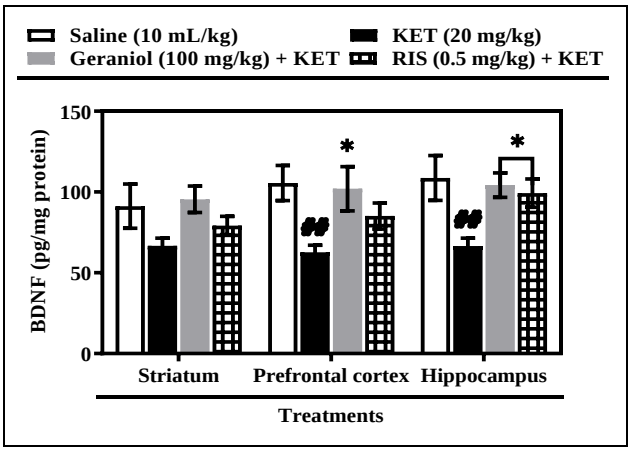

Figure 6: Preventive effect of geraniol in ketamine-induced changes in brain derived neurotrophic factor (BDNF) concentrations in the striatum, prefrontal cortex and hippocampus of mice brains.

Bars represent the mean±S.E.M of 7 animals/group; ##$p<0.01$ compared to saline group; *$p<0.05$ compared to KET group (two-way ANOVA followed by Bonferroni post hoc test); KET=ketamine, RIS=risperidone.

DISCUSSION

The findings of this study show that ketamine (NMDAr antagonist)] induce neurobehavioral patterns in mice that are similar to human psychosis.[6,31,32] Ketamine also deranged neurochemical (DA, 5-HT, GAD, and AChE) and neurotrophic (BDNF) pathways in the striatum, prefrontal cortex, and hippocampus of mice.[4,9] However, the brain insults caused by these psychotomimetics were prevented by geraniol, a naturally occurring acyclic monoterpene with diverse pharmacological activities.[17-19,21,23] To the best of our knowledge, this is the first investigation to establish that geraniol has antipsychotic-like activity in a murine model.

Subanesthetic doses of ketamine cause NMDAR inhibition, which leads to the establishment of hyperdopaminergic pathways in the subcortical regions of the brain (striatum and nucleus accumbens) via reduced GABAergic inhibition.[4] This dysfunctional pathway has been linked to be the pathological basis of positive symptoms of the disease.[33] Remarkably, the administration of geraniol irrespective of the models annulled schizophrenia-like symptoms. Geraniol decreased stereotyped behavior and spontaneous motor activity, both of which are indices of positive symptoms, and it seems to act on both dopaminergic and glutaminergic pathways.

Apart from inducing positive symptoms, ketamine has been shown to alter other neurotransmitters signaling pathways to induced negative and cognitive symptoms largely reported to be associated with schizophrenia symptomology.[34] For instance, hypodopaminergic activity caused by NMDA inhibition in the mesocortical and prefrontal cortex regions of the brain has been linked to the pathogenesis of negative and cognitive symptoms.[4] Also, NMDAR blockade affects the physiology of neurotransmitters such as 5-HT and GABA, which are evidenced to be the core basis for the complexity of these symptoms of schizophrenia that the available pharmacotherapies failed to treat.[35] So, geraniol's ability to alleviate these symptoms in the current study broadened its pharmacological actions as an intervention in many central nervous system conditions such as depression, Parkinson's and Alzheimer's[18] as well as spinal cord injury.[20,24,36,37] Herein, our findings suggest that geraniol elicit anti-psychotic-like action against positive, negative and cognitive symptoms of schizophrenia.

In this study, dopamine levels in the striatum and hippocampus were decreased by geraniol administration as a preventive intervention in the ketamine models of psychosis, while dopamine levels in the prefrontal cortex remained variable because there was a rise and fall in DA's content in the preventive studies. At first, our findings confirm earlier findings that repeated ketamine administration increased dopamine levels in the cortex,

striatum, hippocampus, and nucleus accumbens, it also accords with other reports that dopaminergic activities were suppressed in these brain regions during the treatment of schizophrenia.[4]

Along with the dopaminergic system, GABAergic neurotransmission also plays a role in schizophrenia, and it is a known hypothesis that ketamine NMDAR inhibition causes mesolimbic hyperdopaminergia via GABAergic inhibition. In this study, GAD, an enzyme that catalyzed the biosynthesis of GABA from glutamate, was assessed. We found decreased GAD levels in the various brain regions of ketamine-treated mice as previously demonstrated.[9] Geraniol treatment abated this effect of ketamine, by upregulating the activity of GAD.[9] Notably, the 5-HTergic system is another system that has been linked to schizophrenia, particularly in the pathogenesis of its negative and cognitive symptoms.[32] According to clinical and preclinical studies, prolonged ketamine treatment interferes with the 5-THergic pathway by activating the expression of 5-HT_{2A} in the frontal-parietal cortex as well as increasing the brain level of cortical and striatal 5-HT.[37] Of note, these brain regions have been majorly associated with the onset of complex symptoms of schizophrenia that the typical antipsychotics unable to treat.[32] In the current study, the preventive treatment with geraniol was found to lower the levels of 5-HT in the ST, PFC, and HC altered by ketamine. Here, we suggested that 5-HT_{2A} may also be involved in the mechanism of action of geraniol on SCZ mice showing negative and probably cognitive symptoms, although this has not been elucidated using antagonist receptor interaction study.

In addition to other implicated pathways underlying the pathogenesis of the cognitive symptoms of schizophrenia, repeated ketamine insult is known to also disrupted the cholinergic neurotransmission, an important pathway involved in the regulation of learning and memory formation.[38] In this study, AChE, an enzyme that is responsible for the metabolic degradation of ACh to choline and acetate, was assessed and used as an indicator of the effect of geraniol on ketamine-induced cognitive impairment. Thus, our data showed a decreased activity of AChE in the brain (striatum, prefrontal cortex and hippocampus) of mice exposed to co-administration of ketamine and geraniol in the preventive treatment. Previous studies have shown that the ability of memory enhancing and antipsychotic drugs to improve the cognitive symptoms of schizophrenia is partly and clinically dependent on the extent of inhibition of AChE enzyme activity especially in cortical brains regions.[6,9,32] Therefore, the finding that geraniol inhibits ketamine-induced increased AChE activity, suggests potential ability to ameliorate the cognitive deficits common to schizophrenia disease.

Lastly, it has been observed that schizophrenia is correlated with alteration in neurotrophic growth factor (BDNF), a neurotrophic growth factor that is essential for the growth and survival of neurons, particularly the cholinergic, dopaminergic, and motor neurons.[39] BDNF, which is produced in nerve and glial cells, has been coupled to synaptic transmission, plasticity, learning, and memory formation.[40] Unfortunately, repeated exposure with ketamine, an NMDAR antagonist, has been shown to interfere with this BDNF and lower its levels in various areas of the brain, particularly the hippocampus prefrontal cortex and striatum, thereby leading to cognitive deficits.[6] Geraniol in the current study prevented the decrease in BDNF caused by ketamine in the brain regions. This suggests that consuming a natural acyclic monoterpene isolate from herb oils such like geraniol may be indispensable in the biosynthesis of BDNF and its role in memory formation.

CONCLUSION

In this study, we further confirmed that repeated ketamine intoxication causes neurochemical dysregulations in the cortical and sub-cortical brain regions which are known to underpin schizophrenia-like symptoms. Nevertheless, geraniol treatment prevented these symptoms, which included positive, negative, and cognitive symptoms, by modulating the neurotransmitter and neurotrophic factor pathways. As a result, geraniol may be a promising molecule for the treatment of negative and cognitive symptoms in schizophrenic patients given the current challenges of existing antipsychotic drugs.

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