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Nardostachys jatamansi Targets BDNF-TrkB to Alleviate Ketamine-Induced Schizophrenia-Like Symptoms in Rats

Anjali Janardhanan Anjana Sadanand Arambakkam Janardhanam Van

Department of Biochemistry, University of Madras, Chennai, India

Keywords

Schizophrenia · N-methyl-D-aspartate receptor antagonist schizophrenia-like rat model · Brain-derived neurotrophic factor · Tropomyosin receptor kinase B · Glutamate · Dopamine · *Nardostachys jatamansi*

Abstract

Objective: Schizophrenia, a common neurological disorder appearing in the late teens or early adulthood, is characterized by disorganized thinking, behavior, perception of emotions. Aberrant N-methyl-D-aspartate (NMDA) receptor-mediated synaptic plasticity is a pathological event here due to dysfunction of dopamine and glutamate transmission at NMDA receptors. Dysregulated brain-derived neurotrophic factor (BDNF) i.e., its signalling through the tropomyosin receptor kinase B (TrkB) receptor, is a major feature of schizophrenia. With recent global awareness of traditional plant medicine reducing side effects, the aim of our study was to evaluate the efficacy of the ethanolic root extract of herb belonging to the Valerianacea family, *Nardostachys jatamansi*, against ketamine-induced schizophrenia-like model in rats. ***Methods:*** The effect of the *N. jatamansi* drug (oral dosage of 500 mg/kg body weight for 14 days) in ketamine-administered male Wistar albino rats (30 mg/kg body weight for 5 days) on modulating behaviour and the level of neurotransmitters like dopamine and glutamate was whole-brain homogenates, and its influence on BDNF and TrkB levels in 2 relevant brain regions, the hippocampus and prefrontal cortex, was assessed. ***Results:*** We observed that *N. jatamansi* treatment exhibited encouraging results in the modulation of ketamine-induced schizophrenia-like behaviours, principally the positive symptoms. Our drug both significantly upregulated the glutamate level and downregulated the dopamine level in whole-brain homogenates and retained the normal levels of BDNF (in the hippocampus but not in the prefrontal cortex) and TrkB (in both hippocampus and prefrontal cortex) induced by ketamine in rats. ***Conclusion:*** These findings suggest a neuroprotective effect of the ethanolic root extract of *N. jatamansi* against ketamine-induced schizophrenia-like symptoms in rats; possibly, regarding its effect on TrkB signalling. Further research is warranted in the treatment of schizophrenic symptoms.

Introduction

Schizophrenia, a common neurological disorder, is a brain disorder generally characterized by disorganization of speech and thought. This disorganization results in what is termed as "positive symptoms" (hallucinations, delusions, etc.) or "negative symptoms" (alogia, anhedonia, catatonia, avolition, asociality, etc.) that affect the

Dr. Arambakkam Janardhanam Vanisree
Department of Biochemistry
University of Madras, Guindy Campus
Chennai, Tamil Nadu 600 025 (India)
E-Mail vanielango@gmail.com

way a person acts or thinks or sees the world; most cases of schizophrenia appear in the late teens or early adulthood [1]. Despite all research efforts, there is still no unanimity about its exact pathophysiological mechanisms and the genes involved. However, in the last 2 decades, a large number of neurophysiological and neuroimaging studies of patients with schizophrenia have provided in vivo evidence for dysconnectivity, i.e., an abnormal functional integration of brain processes in the neuropathological complications in schizophrenia [2].

The core pathology of schizophrenia is the aberrant N-methyl-D-aspartate (NMDA) receptor-mediated synaptic plasticity due to abnormal regulation of NMDA receptors by neuromodulatory transmitters like dopamine, serotonin, or acetylcholine [3]. There is also evidence suggesting the dysfunction of glutamate transmission at the NMDA receptor contributing to the negative symptoms and cognitive impairments observed in schizophrenia [4]. As NMDA receptor antagonists can be used for modelling schizophrenia [5, 6], we have chosen one such antagonist, ketamine, a phencyclidine hydrochloride derivative, in our study. Ketamine was proved to induce schizophrenia-like symptoms in rats like hyperlocomotion and social withdrawal, thereby mimicking the glutamate deficiency hypothesis described in the disease [7]. Reports have proven that ketamine induces symptoms of schizophrenia including delusions, thought diso[illegible] and hallucinations when introduced in humans [8].

Brain-derived neurotrophic factor (BDNF) is [illegible] of the major neurotrophic factors that prima[illegible] suppor[illegible] growth and survival of cholinergic, do[illegible]gi[illegible] and motor neurons through its receptor t[illegible]omyosi[illegible]ptor kinase B (TrkB) which is a tyrosine [illegible]ase receptor possessing tropomyosin-related kinase a[illegible]. BDNF is synthesized by neurons in t[illegible]al cortex and hippocampus of rodents [11–13] and [illegible]s synaptic density [14] and cognition lik[illegible]em[illegible]ar[illegible]learning [15] which are the two param[illegible]signi[illegible]y affected in patients with schizo[illegible]renia. [illegible]literature has shown conflicting reports of b[illegible]crease[illegible]decreased levels of BDNF in conditions o[illegible]phrenia. A decrease in BDNF levels in the fro[illegible]rtex[illegible]brain [16] and in plasma [17] of indiv[illegible]als with schizophrenia has been reported. In contrast, [illegible]er[illegible]dy demonstrated an increase in BDNF [illegible]els i[illegible]ppocampus of schizophrenic patients along [illegible]decrease in TrkB in the corticolimbic structure [18]. [illegible]hu[illegible]rkB-BDNF signalling has been implicated in [illegible]hizophrenia in addition to several other neurological [illegible]eases [19] and hence was adopted in the study.

The safety profile of antipsychotic drugs which are in use today against schizophrenia is not so promising considering the side effects which include akathisia, acute muscle dystonia, and tardive dyskinesia, or tardive psychosis [20]. Our present study was a trial for the explora[illegible]tion of a herb which can be used for the treatmen[illegible] schizophrenia, *Nardostachys jatamansi* (N[illegible] which[illegible] longs to the Valerianaceae family found in the [illegible]ala[illegible] region of India [21]. The roots and the rhizom[illegible]acts (ethanolic) and its fraction have been stu[illegible]d for t[illegible] provement of cognition and it[illegible]conv[illegible]ant, antipar-kinson, and hepatotonic activities [22–24[illegible]gh the plant has been studied for various [illegible]ailments, its potential still remains unexpl[illegible]

Our objective was to st[illegible]n prote[illegible]tive activity of ethanolic root extracts [illegible]J ag[illegible]etamine-induced schizophrenia-like sy[illegible]ms, especially locomotor hyperactivity [illegible]to enhan[illegible]dopaminergic action, working memo[illegible]and so[illegible]isolation in rats.

Materials and Methods

[illegible]ation of NJ Extract

Roo[illegible]J were purchased from a local herbal market and were [illegible]ent[illegible]d by Dr. D. Aravind, Asst. Professor, Department of [illegible]ational Institute of Siddha, Tambaram, Tamil Nadu, India. Clean NJ roots were dried and crushed to powder and extracted with 90% ethanol using a Soxhlet extractor. The extract was dried to powder with a vacuum evaporator and stored at 4°C. The percentage yield was calculated as 12% w/w compared to dried matter. This vacuum-evaporated dried powder was reconstituted in 20% Tween-20 and given orally at 500 mg/kg body weight to rats (NJ extract). The dosage was fixed based on earlier literature studies [25].

Animal Studies

Male Wistar albino rats (120–150 g) were procured as per the norms of the IAEC (Institutional Animal Ethical Clearance-360/01/a CPCSEA-IAEC No. 11/01/2014), University of Madras, Guindy Campus, Chennai, from the Kings Institute, Guindy, Chennai. They were maintained at the animal house facility of the University of Madras, Guindy Campus, and acclimatized to animal house conditions, fed commercial pellet rat chow (Hindustan Lever Ltd., Bangalore, India) and water ad libitum. The animals were divided into 4 groups; each group contained 6 animals:

Group 1 – control, received normal saline intraperitoneally for 5 consecutive days

Group 2 – ketamine group (Ket group), received 30 mg/kg body weight of ketamine (ketamine hydrochloride, Sigma-Aldrich, dissolved in physiological saline and prepared fresh) intraperitoneally for 5 consecutive days at 1 mL/100 g body weight

Group 3 – Ket + NJ group, received 30 mg/kg body weight of ketamine intraperitoneally for 5 consecutive days along with a co-treatment of NJ extract orally (gavage) up to a total period of 14 days at 500 mg/kg body weight

Group 4 – NJ group, received NJ extract (500 mg/kg body weight) orally up to a total period of 14 days

Behavioural Studies

The animals were subjected to behavioural analysis on the fourteenth day from the last induction of ketamine (twentieth day from day 1) between 9:00 a.m. and 5:00 p.m.

Open Field Test

At the start of the experiment each animal was placed in the periphery of an open arena (72 × 72 × 36 cm) which was equally divided into 16 squares and allowed to move freely. The locomotive activity of the rats was calculated based on the number of squares crossed (number) and rearing (number) for a period of 1 h. Stereotype behaviours like grooming and sniffing (in seconds) were also analysed manually for a period of 1 h [26].

Y Maze Test

The test procedure consisted of a single 1-h session in a Y maze (50 × 12 × 12 cm) during which all arm entries and their sequence were recorded manually for a period of 1 h. All the limbs entered into an arm were counted as 1 arm entry. The percentage of alteration was calculated using the formula: number of alterations/total number of arm entries – 2 × 100% [27].

Social Interaction Test

The amount of social interaction was measured as the total amount of time spent on social exploration with a new cage mate for a period of 1 h manually in an open field chamber [28].

The animals were euthanized by cervical dislocation after the behavioural studies. The brain was carefully dissected, and different parts – hippocampus and prefrontal cortex (PFC) – were ... cessed for further studies. A whole-brain homogenate was ... dopamine and glutamate analysis as part of primary studi... drug and its effect on the rat model.

Analysis of Dopamine and Glutamate

Dopamine Analysis

50 mg of brain tissue (excluding the c... ellum) was homogenized in 5 mL HCl-butanol (0.85 mL of 3... ydro...loric acid in 1 L *n*-butanol) for 1 min, then centrifuged i... t 2,000 rpm. To 1 mL of supernatant, 2.5 mL ... and ... mL 0.1 M HCl were added, and the mixture ... d at previous conditions and the upper organi... layer ... To 0.2 mL of the bottom aqueous phase, 0.05 ... 0.4 M ... d 0.1 mL of sodium acetate buffer (pH 6. 9) ... d by 0.1 mL iodine solution (0.1 M in ethanol) for o... n w... ded. The reaction was stopped with 0.1 mL Na_2SO_3 ... ion (0.... Na_2SO_3 in 2 mL H_2O + 18 mL 5 M NaOH) after 2... llowed by addition of 0.1 mL 10 M acetic acid after 1.5 mi... The ... was then heated to 100°C for 6 min. When t... again reached room temperature, excitation and emissi... spectra were read from the spectrofluorometer at 330–375 nm.

...tamate Analysis

...f brain tissue (excluding the cerebellum) was homogenized in 5 mL HCl-butanol for about 1 min, then centrifuged for ... min at 2,000 rpm. 1 mL of supernatant was evaporated to dryness and the residue reconstituted in 100 mL distilled water. Chromatography was run using Whatmann No. 1 against 2 mM glutamate as standard using the mobile phase butanol:acetic acid:water (12:3:5). After drying the paper, ninhydrin was sprayed, and the portion that carried glutamate corresponding to standard was eluted with 0.005% $CuSO_4$ in 75% ethanol. The absorbance was read at 515 nm [30].

mRNA RT-PCR Studies

Total RNA was isolated from a known weight ... brain t... using TRIsoln (Merck-Genei), and cDNA was ... ed ... mRNA using a cDNA synthesis kit (Thermo Scientifi... nes ... *GAPDH*, *BDNF*, and *TrkB* were amplified from ... ng ... NA from each group using the following primers (Si...): for ... Fp – 5′-TCTCTGCTCCTCCCTGT...T-3′ a... p – 5′-TA...G-GCCAAATCCGTTCACA-3′, for *B...F* Fp – ...TGT-GTGCTCGCGTT-3′ and Rp – 5′-TGC... ...GGGC-AG-3′, for *TrkB* Fp – 5′-GGGAC...CTATCCC-3′ and Rp – 5′-GTCACAGCTCAC... ...GCA-... CR conditions were 94°C for 2 min, 94°C fo... 2°C fo... 30 s (34 cycles), 72°C for 1 min, 72°C for 3... (fo... *H*); 94°C for 2 min, 94°C for 30 s, 56°C for 3... cycles), ... C for 1 min, 72°C for 3 min (for *BDNF*), and 94... 2 min, 94°C for 30 s, 58.2°C for 30 s (34 cyc... C for 1 ... 2°C for 3 min (for *TrkB*). The products (... PDH, ... bp for BDNF and 104 bp for TrkB) were a... sed ... rose gel, and bands obtained were quantified usi... J s... ware.

...estern Blot A...sis of BDNF and TrkB

...own weigh... of tissue was homogenized with RIPA buffer ...z Biotechnology – Cat. No. sc-24948). Homogenates ...re c... ged and supernatants collected. Protein concentra... w... stimated with the Bradford procedure using bovine ... umin as the standard [31]. TrkB, mature BDNF protein, ... and β-actin from 60 μg of total protein homogenate were analysed ... Western blot with primary antibodies: anti-TrkB (145 kDa), ...nti-BDNF (14 kDa mature BDNF), anti-β-actin (15 kDa) (1:200, Santa Cruz Biotechnology – Cat. No. sc-8318, sc-20981, and sc-130657, respectively), followed by anti-goat IgG horseradish peroxidase (Santa Cruz Biotechnology). Immunocomplexes were visualized by 3,3′-diaminobenzidine, normalized with β-actin (Santa Cruz Biotechnology) and quantified using Image J software.

Immunohistochemistry Studies

Sections of the brain tissue were deparaffinized, hydrated and subjected to antigen retrieval by incubating with hot sodium citrate buffer for 20 min. The slides were washed with Tris-buffered saline (TBS) buffer and blocked for 1 h in 3% bovine serum albumin-TBS. After washing, the slides were incubated with monoclonal antibodies for BDNF and TrkB in 1:100 dilutions overnight at 4°C. Slides were washed with TBS and incubated with anti-rabbit horseradish peroxidase conjugate (Genei-Merck) for 1 h at room temperature. Slides were washed with TBS, followed by 3,3′-diaminobenzidine and mounted with dibutyl phthalate in xylene [32]. The nucleus was counterstained with haematoxylin. Cells with brown appearance in the cytoplasm or cell membrane were considered positive. The intensity of staining (0.5 – weak, 1 – medium, 2 – intense) and the percentage of positive cells (0–5% = 0, 6–50% = 1, ≥51% = 2) were assessed in at least four 400× fields. The score of each slide was multiplied to give a final score from 0 to 4 and was finally determined as negative score 0, very low expression

Table 1. Open field test

	Squares crossed, *n*	Rearing, *n*	Grooming, s	Sniffing, s
Group 1: control	227.83±16.95	48±7.23	233.67±26.59	226.5±25.08
Group 2: Ket	256.17±13.24[a]	65.83±13.84[a]	270.83±18.01[a]	269±17.04[a]
Group 3: Ket + NJ	232.17±16.01[b]	47.5±8.68[b]	234±23.59[b]	232.5±23.96[b]
Group 4: NJ	223.33±15.14[ns]	46.3±12.33[ns]	227.17±20.84[ns]	223.17±0.47[ns]

Assessment made in the open field test, $n = 6$. [a] $p \leq 0.05$ when compared to group 1; [b] $p \leq 0.05$ when compared to group [illegible] non-significant when compared to group 1. Significant levels correspond to Bonferroni-corrected values.

Table 2. Y maze and social interaction tests

	Alteration score, %	Social interaction, s
Group 1: control	44.67±3.64	662±52.71
Group 2: Ket	35.26±3.76[a]	608.17±41.75[a]
Group 3: Ket + NJ	44.27±2.82[b]	655.33±47.77[b]
Group 4: NJ	43.19±6.24[ns]	665.33±47.4[ns]

Analysis of Y maze test and social interaction test, $n = 6$. [a] $p \leq 0.05$ when compared to group 1; [b] $p \leq 0.05$ when compared to group 2; ns, non-significant when compared to group 1. Significant levels correspond to Bonferroni-corrected values.

Table 3. Levels of dopamine and [illegible]mate

	Dopamine, [illegible] tissue	Glutamate, mg/g tissue
Group 1: [illegible]	0.56[illegible]25	0.511±0.10
Group 2: Ket	[illegible]840±0.11[a]	0.335±0.067[a]
Group 3: Ket [illegible]	[illegible]8±0.16[b]	0.495±0.08[b]
Group 4: NJ	0.500±0.14[ns]	0.588±0.11[ns]

[illegible]els of dopamine and glutamate in rat whole-brain homogenates, $n = 6$. [a] $p \leq 0.05$ when compared to group 1; [b] $p \leq 0.05$ when compared to group 2; ns, non-significant when compared to group 1. Significant levels correspond to Bonferroni-corrected values.

score <2, low expression score 2, or higher expression score 4. The person scoring the slides was blind to the experiment.

Statistical Analysis

Results were analysed as means ± standard deviation (SD). Statistical analysis of the data was performed using Student *t* test and one-way analysis of variance. Bonferroni correction was performed, and *p* values ≤0.05 were considered as significant.

Results

Behavioural Studies

Open Field Test

Open field test showed significant hyperlocomotion in the ketamine-induced schizophrenia-like model group 2 (Ket) when compared to that of group 1 (control) as observed in squares crossed by the subject ($p = 0.02$) and frequency of rearing ($p = 0.03$). A similar significant increase in stereotype characters like grooming ($p = 0.05$) and sniffing ($p = 0.01$) was also observed in group 2 rats when compared to those of group 1. Ket + NJ group 3 rats significantly reduced ketamine-induced hyperlocomotion – number of squares crossed ($p = 0.02$), rearing ($p = 0.02$) and stereotype behaviours like grooming ($p = 0.02$) and sniffing ($p = 0.03$) when compared to group 2. No significant variations were observed between group 1 and group 4 (NJ) animals (Table 1).

Y Maze test

The Y maze test to evaluate immediate working memory showed a significantly lower alteration score for group 2 rats when compared to group 1 ($p = 0.01$). However, NJ treatment could significantly enhance the working memory in group 3 rats when compared to group 2 ($p = 0.02$). No significant variations were observed between group 1 and group 4 (Table 2).

Social Interaction Test

The social interaction test was performed to study the effect of NJ on ketamine-induced negative feature-like symptoms of schizophrenia in rats. The ketamine-induced significant reduction in social interaction (p =

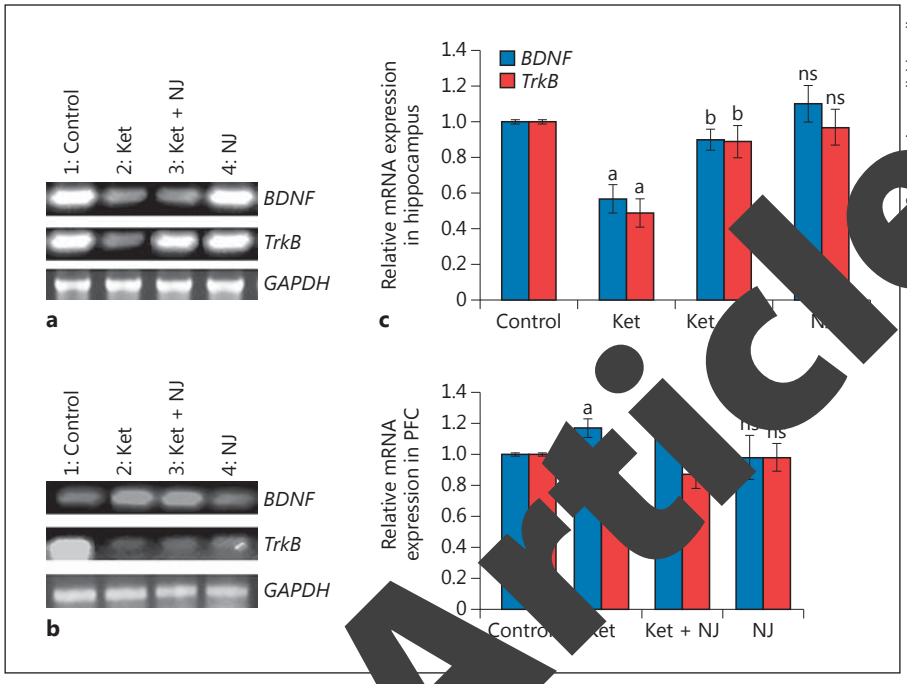

Fig. 1. mRNA expression representations of genes *BDNF* and *TrkB* in 4 experimental groups – control (1), Ket (2), Ket + NJ (3), and NJ (4) – in the hippocampus (**a**) and PFC (**b**). Relative expressions are graphically represented in the hippocampus (**c**) and PFC (**d**). Results are expressed as means ± SD. [a] $p \leq 0.05$ considered significant when compared to group 1; [b] $p \leq 0.05$ considered significant when compared to group 2. ns, non-significant when compared to control (1). [#] Non-significant when compared to Ket (2). $n = 6$. Significant levels correspond to Bonferroni-corrected values.

0.01) in group 2 rats was significantly enhanced by NJ treatment in group 4 ($p = 0.04$) when compared to group 2 (Table 2).

Analysis of Dopamine and Glutamate

Dopamine estimation in brain suggested that [illegible] dopamine level was significantly increased in the ket[illegible] induced schizophrenia-like model grou[illegible] when compared to group 1 ($p = 0.02$). Treatment of [illegible] could significantly reduce dopamine levels [illegible] group [illegible] ($p = 0.02$) when compared to group 2. However, no significant change was observed in group 4 rats [illegible] compared to group 1 (Table 3).

Glutamate analysis exh[illegible] significant reduction in glutamate levels in gr[illegible] 2 [illegible] compared to group 1 ($p = 0.02$). NJ extra[illegible] eated [illegible] 3 rats showed a significant incr[illegible] se in t[illegible] el of glutamate ($p = 0.05$) when compared t[illegible] p 2 ra[illegible] owever, no significant variations were ot[illegible] between groups 4 and 1 in glutamate levels (T[illegible]).

m[illegible]tu[illegible] of BDNF and TrkB

The [illegible] expressions of the genes *BDNF* and *TrkB* [illegible] both the hippocampus and PFC of brains from all the [illegible] perimental groups were analysed. There was a significantly lower gene level of *BDNF* in the hippocampus rats in group 2 compared to group 1 ($p = 0.01$). NJ extract treatment could significantly enhance the level of

[illegible] ($p = 0.04$) [illegible] when group 3 was compared to group [illegible] ver, the level of *BDNF* in group 4 did not show [illegible] y sig[illegible] nt variation when compared to group 1 (Fig. [illegible]).

[illegible] ntrast to the observations made in the hippocampus, the PFC had shown a different pattern of expression for *BDNF*. The gene showed a significantly higher level ($p = 0.02$) in the PFC of group 2 when compared to group 1 while no significant change was observed in group 3 when compared to group 2 ($p = 0.07$). Similar to the previous results, group 4 did not show any variations in expression profile from that of group 1 (Fig. 1b, d).

TrkB, the receptors of *BDNF*, had revealed a different expressional pattern unlike its ligand. The *TrkB* level was significantly downregulated in both hippocampus ($p = 0.01$) and PFC ($p = 0.02$) of group 2 rats when compared to group 1. At the same time, the level of *TrkB* was found to be significantly elevated in the drug-treated group 3 in both the hippocampus ($p = 0.01$) and PFC ($p = 0.03$) when compared to group 2. No significant difference was observed in *TrkB* level in group 4 when compared to group 1 (Fig. 1a–d).

Protein Level of BDNF and TrkB

Western Blot Studies

In the hippocampus, we observed a significant loss in the level of BDNF in group 2 when compared to group 1 ($p = 0.01$). NJ extract treatment could significantly en-

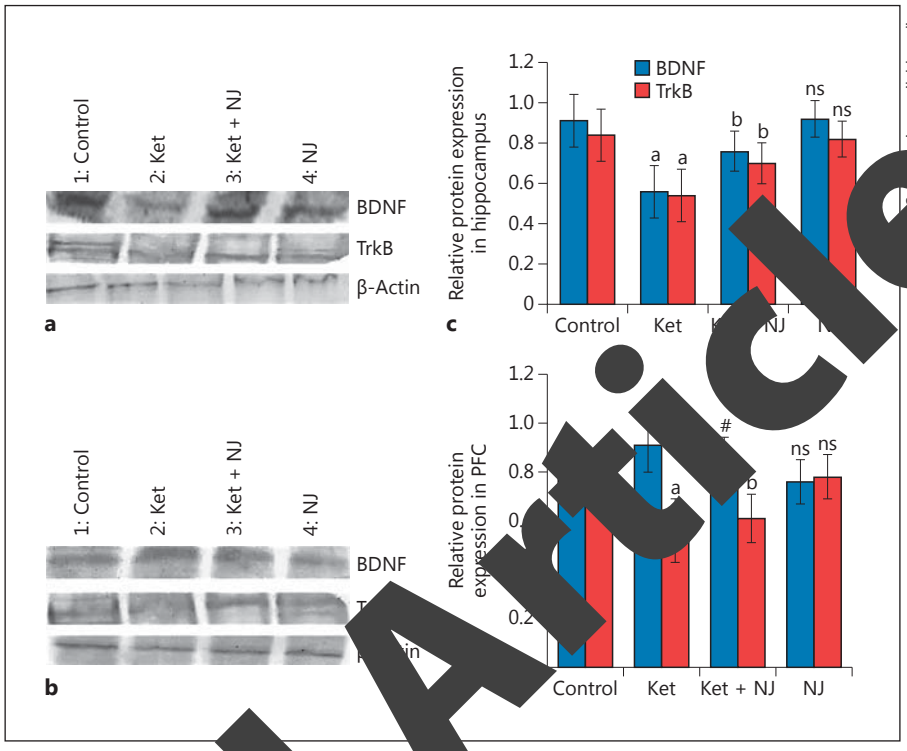

Fig. 2. Immunoblot representations of BDNF and TrkB protein in 4 experimental groups – control (1), Ket (2), Ket + NJ (3), and NJ (4) – in the hippocampus (**a**) and PFC (**b**). Relative expressions are graphically represented in the hippocampus (**c**) and PFC (**d**). Results are expressed as means ± SD. [a] $p \leq 0.05$ considered significant when compared to group 1; [b] $p \leq 0.05$ considered significant when compared to group 2. ns, non-significant when compared to control (1). [#] Non-significant when compared to Ket (2). $n = 6$. Significant levels correspond to Bonferroni-corrected values.

hance the level of BDNF ($p = 0.03$) when group 3 was compared to group 2 (Fig. 2a, c).

In the PFC, we found a significantly higher [le]vel BDNF in group 2 ($p = 0.03$) when compa[red] to g[roup] 1. We could not observe a significant varia[tion] in gro[up] compared to group 2 ($p = 0.09$). In both st[ates] the protein profile of BDNF in group 4 did [not] show [a] variation from the control group 1 (Fig. [2]d).

The expression profile of TrkB sh[owed] similar expression in both the hippocamp[us] and PFC. There was a significant downregulation [of] TrkB [in] both the hippocampus ($p = 0.02$) and PF[C] ($p = 0.03$) in ketamine-induced group 2 rats when c[ompa]red t[o] group 1. NJ extract could significantly [enh]ance [the] level of TrkB in the hippocampus ($p = 0.02$) and PFC ($p = 0.02$) in group 3 animals when compared to [group] 2. No significant variations were observed in [group] 4 [compa]red to group 1 (Fig. 2a–d).

Immunohistochemistry Studies

Similar to our RT-PCR analysis, BDNF in the hippocampus, TrkB in the hippocampus and PFC showed a relatively very low expression (score <2) in group 2 (Ket) animals compared to group 1 (score = 4) based on the number of positive cells for the molecule and intensity of its staining. Group 3 (Ket + NJ) animals in all the above [regions] showed relatively higher expressions of BDNF and TrkB (score = 2) compared to group 2. BDNF expression in the PFC in groups 2 and 3 was comparatively higher (score = 4) when compared to group 1 (score = 2). No variations in score were observed between group 4 and group 1 in any of the cases (Fig. 3).

Discussion

Neurobiological underpinnings of schizophrenia and the related pathways targeted by drugs used in the treatment of schizophrenia remain poorly understood. The change of function in the NMDA receptor was a major criterion in schizophrenia research [6]. The psychotomimetic properties of ketamine and its NMDA receptor antagonist nature have been reported to mimic the features of schizophrenia [7, 34] along with dopaminergic activation causing psychosis [35]. However, the neuropsychiatric mechanisms in this subanaesthetic modelling are still not so clear.

Recently, there has been a global awareness of a traditional system of medication owing to its low side effects, and NJ has already its name mentioned in Ayurveda as a stimulant, antispasmodic, cognition improver,

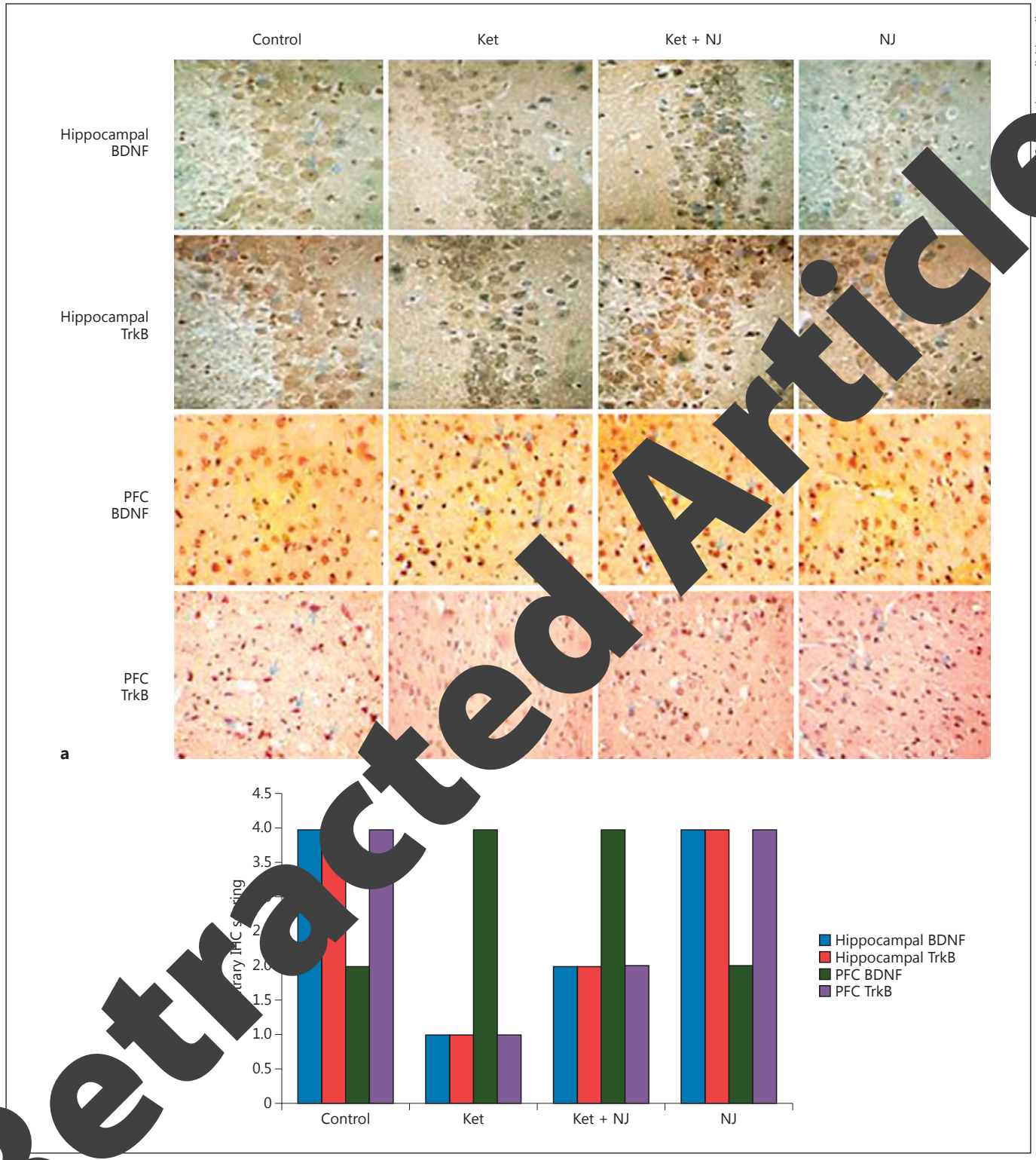

Fig. ...unohistochemistry representations of BDNF and TrkB ... 4 different experimental groups. **a** Photomicrographs of immu... istochemistry expressions of BDNF and TrkB in the 4 experi... ental groups – control (1), Ket (2), Ket + NJ (3), and NJ (4). Magnification ×400. **b** Scoring of each molecule expression in each group is depicted as the mean of $n = 3$. The slides in each group attained identical scores. Score 4 indicates high expression, score 2 low expression, score <2 very low expression, and score 0 nil expression. IHC, immunohistochemistry.

antidepressant, anticonvulsant, and antipsychotic [36, 37]. In the current study, we attempted to analyse its efficacy against the ketamine-induced schizophrenia-like symptoms hyperlocomotion, cognitive impairment, social withdrawal in rats, and its probable mode of action.

Dopaminergic mechanisms mediate locomotor activity, and ketamine may influence its transmission and receptor activation. A pronounced increase in locomotor activity and stereotypic behaviours due to enhanced dopaminergic activity, a major characteristic of schizophrenic rodent models, corresponds to psychomotor agitation observed in schizophrenic patients [28, 38]. This was evident from increased *horizontal* activity (number of squares crossed in an open field), vertical activity (rearing) and stereotype behaviours like sniffing and grooming presented in ketamine-administered group 2 rats [39]. NJ treatment could significantly reduce the hyperlocomotion and stereotypy which is an indication of alleviation of the positive-like symptoms of schizophrenia. Further, spatial memory and cognition defects make one of the major areas of deficit in schizophrenia [40, 41]. We observed a significant elevation in the spontaneous alteration score in the Y maze in NJ-treated group 2 rats, which identifies the ability of the drug to enhance spatial memory. The significant advancement in time for social interaction by NJ treatment is suggestive of the negative symptom-alleviating property of NJ. However, the effectiveness of NJ against negative symptoms of ketamine induced schizophrenia-like symptoms was not as prominent as that of the positive symptoms.

Evidence suggests the involvement of neurotransmitters in schizophrenia, with glutamate and dopamine being important among them [4, 42]. Studies on post-mortem brains of schizophrenics showed significant reduction of glutamate levels in the hippocampus and PFC [43]. Administration of phencyclidine, another NMDA receptor antagonist, to rats was also reported to cause a decrease in glutamate levels due to a shift in the glutamate-glutamine cycle owing to NMDA receptor blockade [44]. The comparative reduction in the levels of glutamate in the brain of rats exhibiting schizophrenia-like symptoms observed in our study reflects the hypofunction of the NMDA receptor compared to that of control. NMDA receptor hypofunction and hence the glutamatergic regulation of subcortical dopaminergic neurons reflects the potential relevance to schizophrenia as discussed by Kegeles et al. [45]. We observed a significant increase in the dopamine levels of group 2 Ket rats similar to a significant increase in dopamine concentration reported in brains of schizophrenic patients [46, 47]. The observed significant increase in dopamine levels in group 2 Ket rats in our study may be contributed by the striatum as an increased presynaptic striatal dopamine synthesis and storage in schizophrenic patients have been reported earlier [48–50]. The reduction of the dopamine level and the increased glutamate level in the brain homogenate of NJ extra[illegible]eated[illegible] are indications of its efficacy, the mechanism[illegible]hich currently unknown. This is suggestive of the po[illegible]l of NJ extract against schizophrenia-like sym[illegible]ms.

Till date, several studies in s[illegible]zophr[illegible] had provided conflicting observations regardin[illegible]BD[illegible] which is an important molecular regulator[illegible]euronal development and plasticity. Some[illegible]s rep[illegible] significantly reduced BDNF levels in th[illegible]n of schizophrenia patients [51] while some re[illegible]ted [illegible] BDNF than normal [52]. The contrad[illegible]y studies project a scenario of, perhaps, a[illegible]regulated[illegible]NF-TrkB pathway in the schizophr[illegible]tion. C[illegible] observations of a reduction in the level[illegible]mR[illegible] proteins of BDNF and TrkB in the hippocam[illegible] ketamine-induced rat schizophrenic [illegible]dels were i[illegible]e with reports showing a decrease in [illegible]F levels [53] and in TrkB levels [18] in the hippo[illegible]f post-mortem brains of schizophrenic patients. [illegible]is i[illegible]s that ketamine administration in rats in sub[illegible]ic dosage could mimic schizophrenia [7, 26] in [illegible]n a decreased BDNF-TrkB signalling in the hippocampal region which could lead to the loss of synaptic plasticity. However, the hippocampal BDNF and TrkB level was elevated in the NJ extract-treated rats, and the modulation of expression may throw some light onto the protective property of NJ. The enhancement of TrkB in NJ-treated rats and probably its downstream factors may, to an extent, lead to the repair of the synaptic dysconnections observed in schizophrenia.

Contrary to our observations in the hippocampus, we found a higher level of BDNF in the PFC of ketamine-administered rat brain when compared to control group 1 rats. Though BDNF is upregulated in the PFC, there is no comparative increase in the expression of its cognate receptor which was found to be downregulated in ketamine-administered rats. We argue that the downregulation of TrkB might be the consequence of upregulation of BDNF as a compensatory measure, and it was shown earlier that the chronic application of BDNF might downregulate its receptor in rats [54]. The downregulation of TrkB in both the PFC and hippocampus observed in our study was supported by similar observations in schizophrenic patients [18]. Thus, in group 2 rats, there exist a disproportionate level of occupancy of receptors with re-

spect to the level of ligand and desensitization of downstream signalling of TrkB in the hippocampus as it is well known that the action of any ligand is dependent on the proportion of receptors occupied by the ligand [55]. Thus, the cumulative effect of an increase in BDNF and the desensitization of TrkB might finally lead to deregulated synaptic connections, a characteristic feature of schizophrenia. Our observations were also supported by Takahashi et al. [18], who stated a loss of ligand-receptor interaction involving BDNF-TrkB as a major feature of schizophrenia. However, the treatment with NJ extract resulted in the enhanced level of TrkB in rats compared to ketamine-induced schizophrenia models suggesting the role of NJ in alleviating the effect induced by ketamine and in turn the schizophrenia-like features. NJ might be exerting its agonistic effect to restore the BDNF signalling which requires further investigations. As immunohistochemistry analysis was scored based on the percentage of positive cells for a molecule and its intensity of staining, our observations of the in situ expression of BDNF and TrkB also supported our results of mRNA and blot analysis.

In summary, our findings reflected the potential of ethanolic root extracts of NJ to attenuate the features of ketamine-induced schizophrenia-like features in rats. The previous literature suggests that the principal components in NJ extract may be jatamansone or jatam [56]. However, we speculate that the effective a our extract is a cumulative action of all rather than the active principle alone and the effect of NJ extract in our experim on the reports that claim and stress extract for addressing ailments rather components separately [57, 58]. We sug may balance BDNF-TrkB , and our findings emphasize the impact of NJ drug on toning the levels of the critical neurotransmitters like glutamate and dopamine which are identified to be dysregulated in schizophrenic patients.

Conclusion

It can be concluded from the above findi has the potential to alleviate schizophren perhaps owing to some of its findings highlight the efficacy of th the positive symptoms of schizophren than the negative sympto of this drug extract and the still be an interesting ar ably the ability to fi signalling a with th tract could new avenues of research in the treatment of

owledgements

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Disclosure Statement

The authors indicate that there is no potential conflict of interest in this study.

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