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Reversal of restraint stress caused dendritic atropy in rats by *Nardostachys jatamansi*

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Hippocampus and prefrontal cortex of brain have distinct role in encoding and retrieval of memories. Uncontrollable stress may influence the structural alterations of limbic brain regions and atropy of neurons mainly in the regions like the prefrontal cortex and hippocampus. *Nardostachys jatamansi* (D. Don) DC (NJE) is a perennial herb and is known for its anti-Parkinson's, hepatoprotective, neuroprotective, hypotensive and anti-diabetic activities. Even though, it is an effective therapeutic intervention for memory impairment but, its effect on the neurons of the hippocampus is not clear. In this context, the effect of NJE on chronic restraint stress induced dendritic atropy in rats was studied. Male Wistar rats underwent 21 days of restraint stress in a close fitting rodent restrainer. In combined treatment groups rats were treated with alcoholic extract of NJE at dosage of 200 mg/kg bw for 21 days along with chronic restraint stress. The dendritic morphology of neurons was studied in Golgi-impregnated sections. Stress produced dendritic atropy significantly increased the dendritic branching and intersections in experimental rats. Interestingly, treatment of stressed rats with NJE extract resulted in the reversal of stress induced the dendritic atrophy. These results demonstrate that atrophy of dendritic neurons caused by chronic restraint stress may be responsible for learning and memory impairment. Co-treatment of rats with NJE showed enhancement in the dendritic branching in the hippocampus. Furthermore, NJE treatment significantly increased superoxide dismutase activity and total antioxidant capacity in frontal cortex, hippocampus and striatum regions of brain. Thus, our findings suggest that NJE is a potential neuroprotector, which might be beneficial in the treatment of stress induced memory impairment.

Keywords: Dendritic atropy, Hippocampus, *Nardostachys jatamansi*, Neuron, Stress, Wistar rats

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Learning is a complex process which involves many parts of the limbic system like the hippocampus, amygdala, basal ganglia, hypothalamus and the cortex[1]. Hippocampus is one of the main neuroanatomical structures orchestrating memory processing[2,3]. Unfortunately, the hippocampus is highly susceptible to psychological insults such as stress. The hippocampus contains high levels of glucocorticoid, estrogen and progesterone receptors, which make it more vulnerable to long-term stress than most other brain areas[4,5]. Humans, who have experienced severe long-lasting traumatic stress, show atrophy of the hippocampus more than of other parts of the brain[6]. Experimental studies by Sunanda *et al.*[7] on rats and mice have shown that stress has detrimental effects on the dendritic cytoarchitecture of the neurons in different areas of the brain including the hippocampus.

Nardostachys jatamansi (D. Don) DC (NJE) is a rhizomatous, herbaceous and most primitive species within the family Valerianaceae[8]. It is extensively used in Indian traditional system of medicine for the treatment of pyschosomatic disorders. It is a critically endangered medicinal plant grows at high altitudes in the alpine and sub-alpine regions of the Himalayas and is commonly known as Indian spikenard. Its medicinal use is well-recognized in the Indian, Chinese, Bhutanese, Nepalese, Japanese and Tibetan medicine[9]. The medicinal value of this plant is due to the presence of sesquiterpenes and coumarins as active components. Jatamansone and jatamansin were found to be the major pharmaceutical constituents of ethanolic extracts of NJE rhizomes[10-12]. The root of NJE is well-known for its hepatoprotective, anti-Parkinson, antioxidant effects and for modulation of haematopoietic system[13-17]. Our previous study has given some insight into the protective effects of NJE

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on chronic-restraint stress induced impairments on hippocampus-dependent learning and memory behavior in rats. Wherein, the protection exerted by NJE was mainly through increasing the activity of acetylcholine esterase in the hippocampus[18,19].

There are no experimental studies documented till date, showing the effect of NJE treatment on morphological changes of CA3 pyramidal neurons of hippocampus. Therefore, the present study was carried out to investigate the effect of NJE on morphological changes of dendrites.

Materials and Methods

Animals and experimental design

Inbred Wistar strain male rats aged between 60-80 days, weighing 180-200 g from the institutional animal house, KSHEMA, served as subjects. The rats were housed in a 12 h light/dark environment with *ad libitum* access to water and food (Amruth feeds, India) except during restraint stress procedure. All procedures were performed in strict compliance with the Institutional Animal Ethics Committee (IAEC) approved experimental protocols (Approval letter No. KSHEMA/AEC/044/2006, dated 18-09-2006). The animals were divided into 4 groups (n=6): Group A: Normal control (NC): Non-restraint rats did not undergo any stress procedure; Group B: Restraint stress (RS): subjected to 21 days RS by placing the rats in a close fitting rodent restrainer for 6 h/day (from 10.00 – 16.00) for 21 days. After being restrained, rats were released back into their cages immediately; Group C: Vehicle control (VC): received vehicle (Alcohol + Tween 80 + Water-1:2:5) for 21 days; Group D: Stress with NJE (RS+JM): received 200 mg of NJE/kg body weight of rat for 21 days orally with restraint stress

Nardostachys jatamansi ethanolic root extraction and dose selection

The roots were purchased from a recognized, licensed supplier and were identified and authenticated by the same (Amsar Private Ltd, Indore, India). The cleaned, air dried and finely powdered NJE roots were extracted with 95% ethanol through soxhlet apparatus for 6-8 h. The extract was evaporated to dryness under reduced pressure and temperature using rotary vacuum evaporator, and dried residue was stored at 4°C[14]. The LD_{50} value for NJE in rats was found to be greater than 5000 mg/kg bw[20]. Based on our earlier work[19] the most effective dose of NJE 200 mg/kg bw was selected for this study.

Morphological study of hippocampal CA3 neurons

Morphological study was carried out after sacrificing rats belonging to four groups A-D (normal, stress, vehicle control and stress in combination with NJE). Brain were removed and processed for Golgi staining to do morphometric analysis of neurons. Tissues were processed according to the standardized method of Rao *et al.*[21]. Tracings of the hippocampal CA3 neurons were made using camera lucida. The Sholl's method[22] was used for dendritic scoring dendritic branching points and intersections. Dark neurons located in the CA3 regions which had consistent silver impregnation and had dendritic branching which was not truncated were chosen for quantification.

Estimation of superoxide dismutase

Superoxide dismutase (SOD) activity in the frontal cortex, hippocampus and striatum of rat brain was determined by employing the method of Beauchamp and Fridovich[23]. SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The amount of enzyme which causes 50% inhibition in photochemical reduction of NBT was considered as one enzyme unit.

Estimation of total antioxidant capacity

The total antioxidant capacity (TAC) of NJE was assessed as per the method described by Manuel and Miguel[24]. The TAC was expressed as the absorbance of the sample at 695 nm. The higher absorbance value indicates higher antioxidant activity.

Statistical analysis of data

Data obtained from all the above experiments were correlated and analyzed using repeated measures two way Analysis of Variance (ANOVA), One way ANOVA, whichever was applicable. Further multiple comparisons between the groups were made using the Bonferonni correction factor or by Tukey's test using statistical software package, GraphPadIn Stat version 5. Differences with value of 0.05 or lower were considered to be statistically significant. The significance of the obtained results was designated as $^{*}p<0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$.

Results

Morphology of hippocampal CA3 neurons in rats treated with NJE

The photomicrographs and camera lucida tracings of CA3 pyramidal neurons from different groups of

rats are depicted in (Fig. 1 and Fig. 2), respectively. A decrease in the number of apical dendrites was observed in stressed rats (Fig. 1C and Fig. 2C) compared to control group (Fig. 1A and Fig. 2A). Further, NJE treatment for 21 days ameliorated the dendritic atrophy in stressed rats as noticed from Fig. 1D and Fig. 2D. VC group did not exhibit any significant change in the morphology of hippocampal CA3 neurons when compared to the control group (Fig. 1B and Fig. 2B).

Apical dendritic intersections

In stressed rats compared to the normal group, the number of apical dendritic intersections beyond 40 µm distance from soma was found to be decreased. There was no difference in the number of apical dendritic intersections in any of the segments of VC group in contrast to NC group. As depicted in Table 1, a significant increase ($p<0.001$) in apical dendritic intersections of CA3 neurons in RS+JM treated group from 60 µm to 120 µm distance from the soma was observed compared to RS group.

Apical dendritic branching points

As noticed from Table 2, no such difference was observed in the number of apical dendritic intersections in VC group when compared to the NC group in any of the segments. In contrast, a significant decrease ($p<0.001$) in the number of apical dendritic branching points in RS group was seen at 100 µm and 120 µm segments. However, NJE 200 mg co-

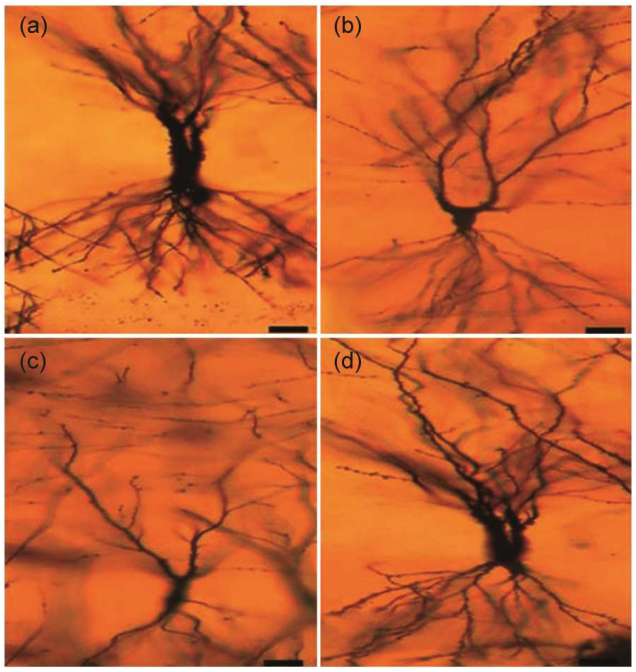

Fig. 1 — Golgi-impregnated CA3 pyramidal neurons of the rat hippocampus from (A) normal control (NC); (B) Vehicle control (VC) (C) Restraint stressed (RS) (D) Stressed with NJE extract (RS+JM)

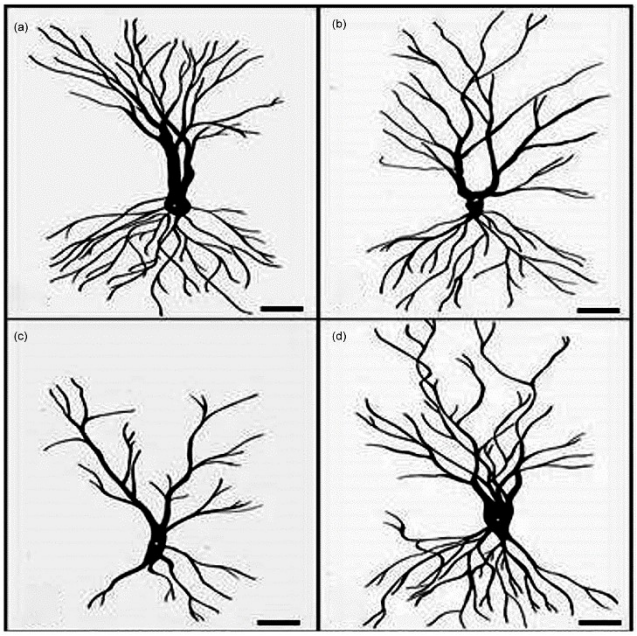

Fig. 2 — Camera lucida tracings of CA3 pyramidal neurons of the rat hippocampus from (A) Normal control (NC); (B) Vehicle control (VC) (C) Restraint stressed (RS) (D) Stressed with NJE extract (RS+JM) Note decreased number of dendrites in RS (C) groups compared to NC (A), VC (B) and RS+JM (D) groups of rats, respectively. Scale bar = 20 µm

Table 1 — Effect of *Nardostachys jatamansi* extract on apical dendritic intersections of hippocampal CA3 neurons of rats.

Group	Distance from soma (µm)					
	20	40	60	80	100	120
NC	1.725±0.0736	2.571±0.1270	4.238±0.1698	5.383±0.2315	5.608±0.3221	3.483±0.1887
VC	1.561±0.1034	2.823±0.1734	4.156±0.2282	5.626±0.6382	6.166±0.4297	3.666±0.3285
RS	1.321±0.0571	1.035***±0.2060	2.263**±0.2499	2.913**,***±0.3357	1.708***±0.2809	1.075±0.2445
RS+JM	1.92±0.1849	3.135±0.2605	7.188$^{###}$±0.4965	9.021$^{###}$±0.2195	9.558$^{###}$±0.2561	8.425$^{###}$±0.3461
ANOVA F	4.8	22.157	42.444	40.529	95.596	117.22
P	NS	<0.001	<0.001	0.001	0.001	0.001

[Values are Mean±SE, n=6. NS=Not significant ***p<0.001 NC, VC, RS+JM>RS. **p<0.01 NC, VC> RS. ###p<0.001RS+JM>NC, VC, RS (One way ANOVA, Bonferonni's test)]

treatment increased ($p<0.001$) the apical dendritic branching points of CA3 neurons from 60 µm to 120 µm distance from the soma when compared to RS group.

Basal dendritic branching points

Analysis of the number of basal dendritic branching points in RS group showed a significant decrease ($p<0.001$) in 20, 40, 60 and 80 µm segments when compared to NC group. There was no difference in the number of basal dendritic branching points in VC group as compared with in any of the segments of normal group. As shown in Table 3, a significant increase ($p<0.001$) in basal dendritic intersections of CA3 neurons in RS+JM group was observed when compared with stressed group.

Basal dendritic intersections

The results of basal dendritic intersections are depicted in Table 4. A decrease ($p<0.001$) in the number of dendritic intersections in the stressed rats was noticed when compared to the 20 to 100 µmsegments of normal control group. However, no such difference was observed in the VC group when compared to the normal group in any of the segments. The test results of restraint stress+NJE showed a significant increase ($p<0.001$) in basal dendritic intersections of CA3 neurons from 20 µm to 100 µm distance from the soma when compared with the stressed group.

Total apical dendritic length

After 21 days of restraint stress a significant decrease in the total apical dendritic length ($p<0.01$) in hippocampal CA3 pyramidal neurons was seen as compared with control group (Fig. 3). All neurons of the restraint stressed group showed atrophy of their apical trees, but detectable changes were observed in the morphology of the apical dendritic trees of the neurons in the rats treated with 200 mg/kg of NJE for 21 days. The length of the apical dendrites in NJE co-treated group was significantly longer than the restraint stressed rats. No significant difference was seen in the length of the neurons of the rats treated

Table 2 — Effect of *Nardostachys jatamansi* extract treatment on rat apical dendritic branching points of hippocampal CA3 neurons

Group		Distance from soma (µm)					
		20	40	60	80	100	120
NC		0.0283±0.0130	0.366±0.611	1.523±0.2152	2.023±0.1703	3.023±0.1798	4.391±0.1375
VC		0.0333±0.0166	0.325±0.0588	1.416±0.1236	2.25±0.2327	3.083±0.2555	4.083±0.2205
RS		0.0616±0.0286	0.11±0.0464	1.195±0.2085	1.361±0.3027	1.64**,***±0.1877	0.978***±0.2588
RS+JM		0.0166±0.0105	0.465±0.1512	3.211$^{###}$±0.2028	4.045$^{###}$±0.264	6.045$^{###}$±0.2374	7.711$^{###}$±0.3514
ANOVA	F	1.06	2.79	23.5	21.5	72.7	117
	P	NS	NS	<0.001	<0.001	<0.001	<0.001

[Values are Mean±SE, Number of rats in each group n=6.NS = Not significant. ***p<0.001 NC, VC, RS+JM>RS. **p<0.01NC, VC>RS. ###p<0.001 RS+JM>NC, VC, RS. (One way ANOVA, Bonferonni's test)]

Table 3 — Effect of *Nardostachys jatamansi* extract treatment on basal dendritic branching points of hippocampal CA3 neurons of rats

Group	Distance from soma (µm)				
	20	40	60	80	100
NC	19.598±0.7756	1.135±0.1628	1.941±0.144	2.023±0.1703	0.926±0.1074
VC	18.416±0.8451	1.173±0.1524	2.008±0.1091	2.25±0.2327	1.383±0.1364
RS	5.473***±0.7764	0.498*±0.0851	1.136 *±0.2319	1.361±0.3027	0.25*,***±0.0645
RS+JM	35.123$^{###}$±0.1839	1.278$^{##}$±0.159	3.831$^{###}$±0.2342	4.045$^{###}$±0.264	2.261$^{###,##}$±0.2368

[Values are Mean±SE. n=6. NS = Not Significant. ***p<0.001 NC, VC, RS+JM>RS. **p<0.01NC, VC>RS. ###p<0.001RS+JM>NC, VC, RS. *p<0.05 RS<NC, VC. ##p<0.01 RS+JM> RS (One way ANOVA, Bonferonni's test)]

Table 4 — Effect of *Nardostachys jatamansi* extract treatment on basal dendritic intersections of hippocampal CA3 neurons of rats

Group	Distance from soma (µm)				
	20	40	60	80	100
NC	2.665±0.519	3.831±0.1658	5.998±0.3008	2.771±0.210	1.896±0.2069
VC	2.856±0.139	3.69±0.2328	5.856±0.2944	2.756±0.263	1.728±0.2259
RS	1.76**,***±0.2202	1.75***±0.2102	2.783***±0.3494	0.968***±0.2108	0.513**,***±0.1101
RS+JM	3.473##,###±0.1502	4.306##±0.169	9.145###±0.2332	4.243###±0.1807	2.253###±0.2175

[Values are Mean±SE, number of rats in each group n=6. NS = Not Significant. ***p<0.001 NC, VC, RS+JM>RS. **p<0.01 NC, VC>RS. ###p<0.001RS+JM>NC, VC, RS. *p<0.05 RS<NC, VC. ##p<0.01 RS+JM> RS (One way ANOVA, Bonferonni'stest)]

with the vehicle in comparison with the normal control group.

Total basal dendritic length

Chronic restraint stress for 21 days caused retraction of the neurons ($p<0.01$) and resulted significant change in the morphology of the dendrites in the basal region. The basal dendrites in these stressed rats were shorter by almost 25% when compared to the normal and VC groups. Treatment of stressed animals with 200 mg of NJE enhanced the growth of the neurons significantly ($p<0.001$). NJE treated rats with stress had basal dendrites which were almost 20% longer than the normal rats as seen in the (Fig. 4).

Effect of *Nardostachys jatamansi* extract and stress on the antioxidant activity in the rat brain

The restraint stress caused changes in activity of SOD in different regions of brain are summarized in Table 5. The highest SOD activity was recorded in the frontal cortex and the lowest in the striatum in the rat brain. As seen in Table 5, in the normal rats the SOD activity was two and half and three folds more in the frontal cortex when compared to the hippocampus and the striatum, respectively. Restraint stress for 6 h per day for 21 days brought about a significant ($p<0.05$) reduction in the SOD activity in some brain regions in the rats compared to the controls. After exposure to restraint stress the frontal cortex depicted the highest decrease in SOD activity in restraint stressed rats. The level of SOD activity was more than three times lesser in this region in stressed rats when compared to the normal group. After treatment with 200 mg of NJE there was considerable elevation in the level of SOD activity in all the three regions of the brain. The SOD activity levels were almost same in 200 mg NJE treated rats and the normal rats in the hippocampus and striatum.

The total antioxidant capacity in the three brain regions is given in Table 6. The highest mean total antioxidant capacity in the frontal cortex region was found to be significantly higher ($p<0.05$) than that in striatum. Further lowest total antioxidant capacity was seen in the hippocampus. Restraint stress for 21 days

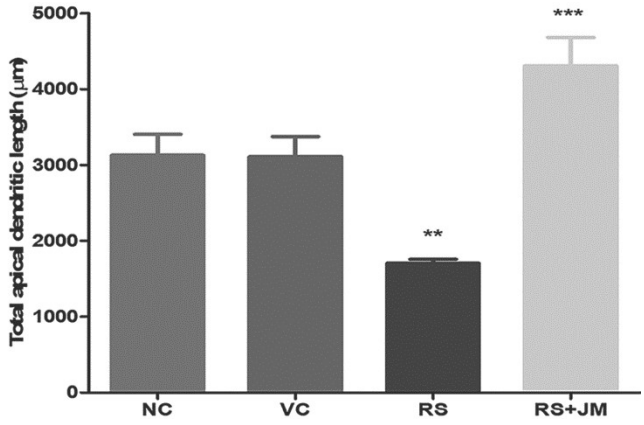

Fig. 3 —Apical dendritic length in hippocampal CA3 neurons of rats treated with *Nardostachys jatamansi* extract. NC: normal control, VC: vehicle control, RS: restraint stress, RS+JM: restraint stress+*N. jatamansi* extract 200 mg. [Values are in Mean±SE. One-way ANOVA: $p<0.001$. Bonferroni adjusted pair wise comparisons: **$p<0.01$ RS<NC, VC; ***$p<0.001$ RS+JM>RS]

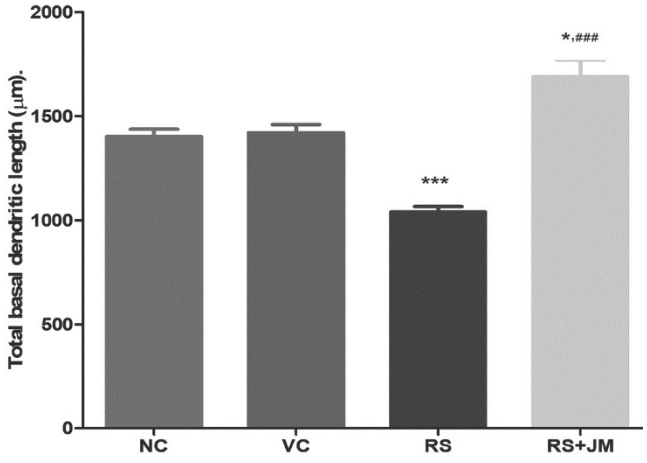

Fig. 4 —Comparison of length of basal dendrites of hippocampal neurons of rats treated with *Nardostachys jatamansi* extract. [Values are in Mean±SE. NC: normal control, VC: vehicle control, RS: restraint stress, RS+JM: restraint stress+*N. jatamansi* extract 200 mg. One-way ANOVA: $p<0.001$. Bonferroni adjusted pair wise comparisons: ***$p<0.01$ RS<NC, VC; ###$p<0.001$ RS+JM>RS; *$p<0.01$ RS+JM>NC, VC]

Table 5 — Effect of *Nardostachys jatamansi* extract on SOD activity in different brain regions of stressed rats.

	SOD activity		
Brain regions	Normal Control (NC)	Restraint stress (RS)	Stress+ NJE (RS+JM)
FrontalCortex	3.7713±0.190	1.0113±0.133*	2.135±0.224** $^{@}$
Hippocampus	1.3612±0.186	0.7550±0.246	1.1375±0.161
Striatum	1.1675±0.126	0.6913±0.061*	1.115±0.158

[Values expressed in Units/mL (n=8); the mean ± SE values of the SOD activity in different brain regions.*denotes $p<0.05$ significant; *Post-hoc* Tukey's test: *$p<0.05$: NC>RS; **$p<0.05$: NC>RS+JM; $^{@}p<0.05$: RS+JM>RS]

Table 6 — Effect of *Nardostachys jatamansi* extract on total antioxidant capacity in different brain regions of stressed rats.

	Total Antioxidant Capacity		
Brain regions	Normal Control (NC)	Restraint stress (RS)	Stress + NJE (RS+JM)
FrontalCortex	143±3.24	101±2.67*	138±2.43$^{@}$
Hippocampus	127±2.50	99±2.442*	124±1.96$^{@}$
Straitum	132±2.330	111±2.307*	116±1.909$^{\#}$

[The mean ± SE of the total antioxidant capacity in different brain regions. Values expressed in (µg/mL) (n=8);* denotes $p<0.05$ significant, *Post-hoc* Tukey's test:*$p<0.05$: NC>RS; $^{@}$ $p<0.05$: RS<RS+JM; $^{\#}p<0.05$: NC>RS+JM]

caused decrease in total antioxidant capacity in all the three regions. Whereas, the frontal cortex and striatum manifests restraint stress-dependent decreases in both SOD ($p<0.05$) and total antioxidant capacity ($p<0.05$) activities. The hippocampus manifests a statistically significant decrease only in the total antioxidant capacity ($p<0.05$). Treating stressed animals with 200 mg of NJE caused significant elevation in the total antioxidant capacity in all the three brain regions.

Discussion

The hippocampal neurons play a major role in functioning of limbic system. Chronic stress exposure has great impact on structure and function of neurons particularly in the hippocampus[25]. Structural and functional damage to the nervous system can cause behavioural changes[26]. It may induce alteration in the dendrites showing increase/decrease in dendritic length and dendritic branches, thus resulting in behavioural alteration[27]. In the present study, our aim was to investigate the effects of NJE on chronic stress induced dendritic atrophy in rats. After daily restraint stress for 21 days we observed that stress induced dendritic atrophy of hippocampal CA3 pyramidal cells in rats. Data presented in Tables 1 and Table 2 on treatment of stressed rats with 200 mg/kg NJE for 21 days showed increase in the dendritic branch points and intersections of the apical and basal regions. The enhanced modelling of the neuronal dendritic morphology in NJE treated rats indicates its positive influence on the circuitry of the neurons, which may provide the basis for functional plasticity. So the increase in the dendritic branches in hippocampal CA3 neurons may result in alterations in synaptic connectivity. This in turn may result in alteration in learning and memory leading to enhancement of cognitive abilities[28].

Glucocorticoids and adrenal steroids which are secreted during stress have hippocampus as their principal neural target. Studies carried out by Moghaddam *et al.*[29] showed that glucocorticoids are responsible for glutamate accumulation following stress in the hippocampus and prefrontal cortex. Thus, enhanced glutamatergic transmission might be responsible for the atrophy of dendrites in CA3 neurons. It is seen in our study that dendritic arborization decreases in stressed rats. In such a condition when rats are treated with NJE there is recovery of the neuronal dendritic tree as seen in Figure 1. The increased numbers of dendritic intersections and branches of hippocampus CA3 neurons may have profound effects on behaviour because of the additional dendrites that are available on these neurons for the formation of new synapses. NJE also indicates its ability to help in repair and restoration of functional neural circuitry in regions of brain affected by stress. Table 1 and Table 2 shows that all CA3 neuron subtypes in restraint stressed rats have a lower number of apical dendritic branch points and lesser apical dendritic intersections as compared to normal control group. The same observation was noted in the basal dendrites (Table 3 and Table 4). Thus, pyramidal neurons from stressed rats appear to retract their dendrites, a change that might have an impact on the total number of dendritic synapses. As shown in our previous study glucocorticoids are clearly elevated in stressed[18] group and are likely to contribute to the atrophy in the hippocampus. This is in concordance with a study by Conrad *et al.*[30] which shows that dendritic remodelling is characterized by a reversible shortening and debranching of apical dendrites. The impairment in learning and memory is associated with alterations in the hippocampal morphology-like retraction of the apical dendrites in the CA3 region of the hippocampus. The changes in cognition can also be associated with changes in the hippocampal dendritic spine number and shape[31].

This is the first study in which the effect of NJE was evaluated on restraint stress-induced oxidative damage in different parts of brain (frontal cortex, hippocampus and striatum). Among various antioxidant mechanisms in the body, SOD is thought

to be one of the major enzymes which protects against tissue damage. They are scavenger enzymes that are reported to work together to eliminate toxic free radicals[32]. In the present investigation, a significant regional variation was observed in the activities of SOD and TAC among frontal cortex, hippocampus and striatum parts of the rat brain. There was uniformity in the pattern of regional variation. When hippocampus and striatum showed low activity of SOD and TAC, high activity was recorded in the frontal cortex. It is interesting to note that these defense elements showed a similar pattern of regional variation in the rat brain. On the other hand, the activity of SOD and TAC were decreased in the stressed rats when compared to control group. This is in agreement with other studies which show that stress causes decrease in antioxidant defense system. Oishi *et al*.[33] have reported that restraint stress results in the imbalance of antioxidant status which ultimately leads to increased oxidative stress thereby resulting in oxidative damage. Stress due to immobilization causes oxidative damage to the hippocampus[34]. Table 5 shows that the activity of SOD decreased significantly ($p<0.05$) in the frontal cortex and the striatum on exposure to restraint stress. There is more than 50% reduction in the activity of SOD in the brain regions on exposure to chronic restraint stress. Furthermore treatment with 200 mg/kg of NJE for 21 days elevated the levels of SOD activity and TAC significantly. The possible reason for decreased activity of SOD and TAC could be more production of free radicals in the stress condition[35,36].

The protective effects offered by NJE may be due its ability to scavenge free radicals that are produced during the stress exposure[37]. Their scavenging efficiency depends on the concentration of phenol and the number and location of the hydroxyl groups[38,39]. Lyle *et al.*[40] have shown that NJE contains large amounts of flavonoids and polyphenols that are responsible for its antioxidant property. These natural antioxidants play a significant role in improving overall health by protecting body from oxidative stress and related disorder[41]. The components present in NJE also processes antimicrobial, hypotensive, anticonvolusant, hepatoprotectve, antidiabetic, and antiarrhythmic activities[42]. In addition to this, a study conducted by Razack *et al.*[43] showed the anxiolytic effects of NJE in mice. Here, NJE offered protection against behaviour anxiolytic action through increasing the brain monoamine and GABA neurotransmitter levels. The extensive use of roots and rhizomes of NJE for a range of pharmacological studies is also evident[9]. The studies suggest that both of the plants exhibit distinctive properties and that their similar therapeutic uses may be dependent on synergistic effects exhibited by the different compounds present in them[44].

Conclusion

The present study suggests that oral intubation of NJE extract may emerge as a useful neuroprotector against the stress-related changes in hippocampal function and structure. This may be hypothesized that these neuroprotective effects is due to the balance between reactive oxygen species and antioxidant system by specific molecules present in it. Results indicated that, reversal of restraint stress caused changes in antioxidant enzyme activity and total antioxidant capacity in various regions of brain by NJE. A significant regional variation was observed in SOD activities and total antioxidant capacity among frontal cortex, hippocampus and striatum regions of the rat brain. Whether the regional variation in the activities of defense enzymes in rat brain correlate with metabolic activities of that particular region is open for further analysis. From this study, it may be concluded that NJE alcoholic root extract treatment may be of value in certain stress-related memory disorders and to improve learning and memory in normal individuals.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

GK and KBS conceptualized the idea. GK performed, analysed and interpreted the data. GK and SS drafted the manuscript. KBS supervised and critically reviewed the manuscript. All authors read and approved the final manuscript.

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