Neuroprotective Potential of *Tinospora cordifolia* in Attenuating Hippocampal CA3 Neuronal Damage in Pregnant Wistar Rats and their Neonates Exposed to Prenatal Vibration Stress and Maternal Separation

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ABSTRACT

Background: Prenatal stress detrimentally impacts cognition, behavior, and psychosocial traits. *Tinospora cordifolia* (TC) is known for its antistress and cognitive enhancement properties. However, its effectiveness against stress induced by maternal separation and vibration is not well-documented. The purpose of the study was to assess the neuroprotective effects of TC on neonatal rats who have experienced the prenatal vibration stress and also stress caused by maternal separation.

Methods: Pregnant Wistar rats in the stressed group experienced three hours of daily vibration stress from 7-16 days of gestation. The treatment group was given 6 mg/kg of TC extract before vibration stress. The neonates were separated from their mother and treated with TC postnatally. At the end treatment period, the rats were subjected to spatial learning task. Following this, animal brains were processed for Golgi cox staining to study the CA3 neuronal arborization.

Results and conclusion: TC-treated mothers showed significantly better spatial learning than those subjected to vibration stress alone. Neonates exposed to prenatal stress took longer time to find the target quadrant, indicating impaired spatial memory, which improved with TC treatment. Increased dendritic branching in CA3 neurons was observed in both TC-treated mothers and neonates. TC extract improves the spatial learning in rats by attenuating the hippocampal CA3 neural damage induced by prenatal vibration stress and maternal separation.

Keywords: Prenatal stress, herbal extract, spatial learning, hippocampus, Tinospora cordifolia.

1. INTRODUCTION

Complex environmental factors impact neurogenesis during the neonatal period, which may impair the limbic brain regions responsible for memory and learning. It is known that prenatal stress raises corticosterone levels in mothers, which suppresses the immune system, results in psychosocial disorders, and alters the morphology of the neurons in brain regions responsible for learning and memory in the offspring [1,2] . Certain *medhya* drugs used in *Ayurveda* have proven to protect neurons from oxidative stress and enhance the cognition in animals with memory deficit by significantly enhancing the dendritic

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architecture of neurons in CA3 sub- region of hippocampus. *Tinospora cordifolia* (TC) is one such *medhya* drug that has antistress, antiulcer, anti-oxidant, immunomodulator and memory and learning enhancing qualities [3,4]. Numerous bioactive compounds with antioxidant and free radical scavenging qualities have been isolated from *Tinospora cordifolia* [5,6].

We hypothesize that treatment with TC will counteract the structural changes in hippocampal CA3 neurons induced by prenatal vibration stress and maternal separation in rat neonates by enhancing dendritic network and improving hippocampus-dependent spatial learning. Hence, the objective was to determine the neuroprotective prospective of TC against hippocampal neuronal plasticity and behavioural impairment induced by prenatal vibration stress and stress caused by postnatal maternal separation in rats.

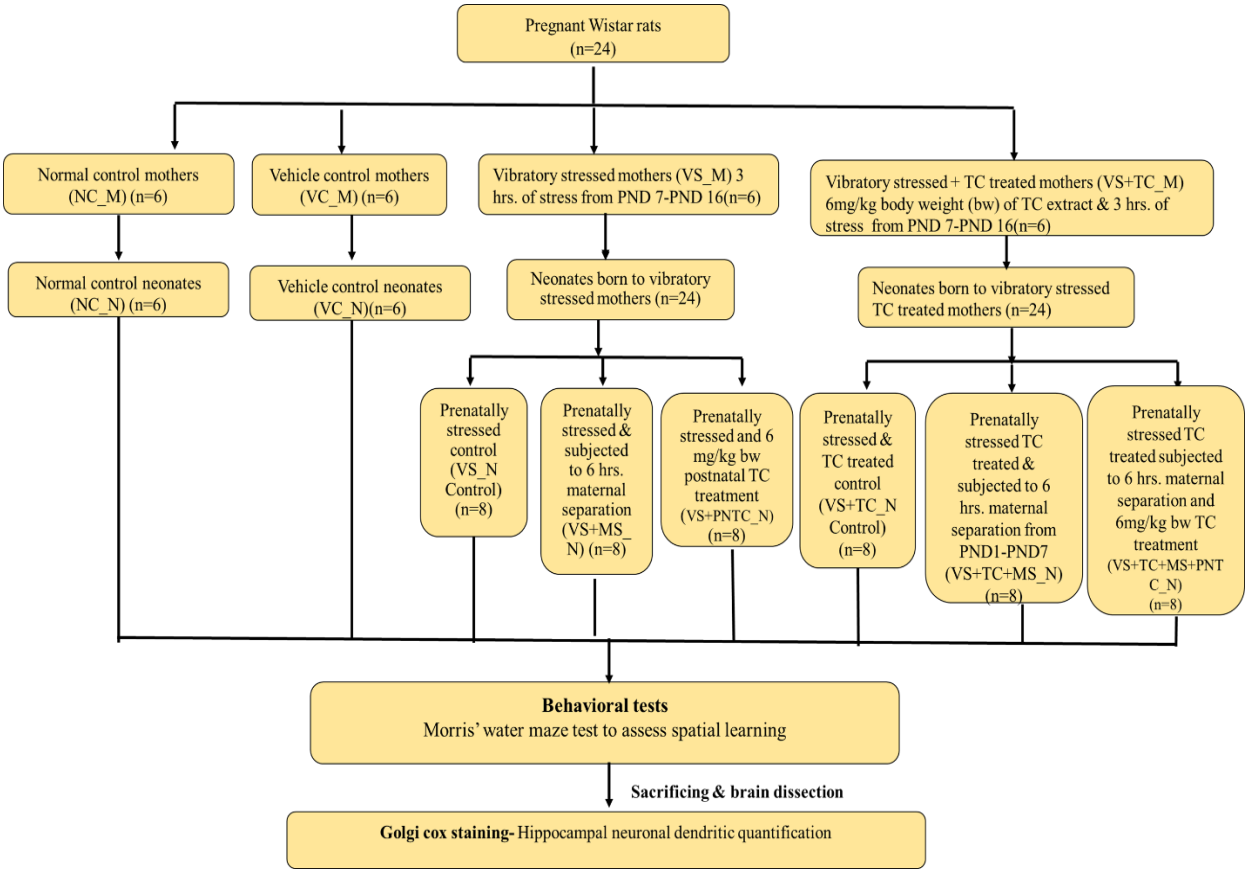
2. EXPERIMENTAL METHODS

2.1 Animals used

Inbred albino female Wistar strain rats were used in the study. The rats were maintained at 29±3° C temperature in 12 hours light and dark cycle and and at humidity (50%-60%) controlled environment with standard food and water. Animal Ethical Committee of our institution gave the approval (IAEC/KMC/56/2015) for this study.

2.2 Study groups

Animals were divided into 4 groups of dams (Figure 1). The rats were bred housing two female and one male adult rats in the same cage for 24 hours. The day of appearance of predominant nucleated epithelial cells in the vaginal smear of female rats was considered as the pre-ovulatory day.



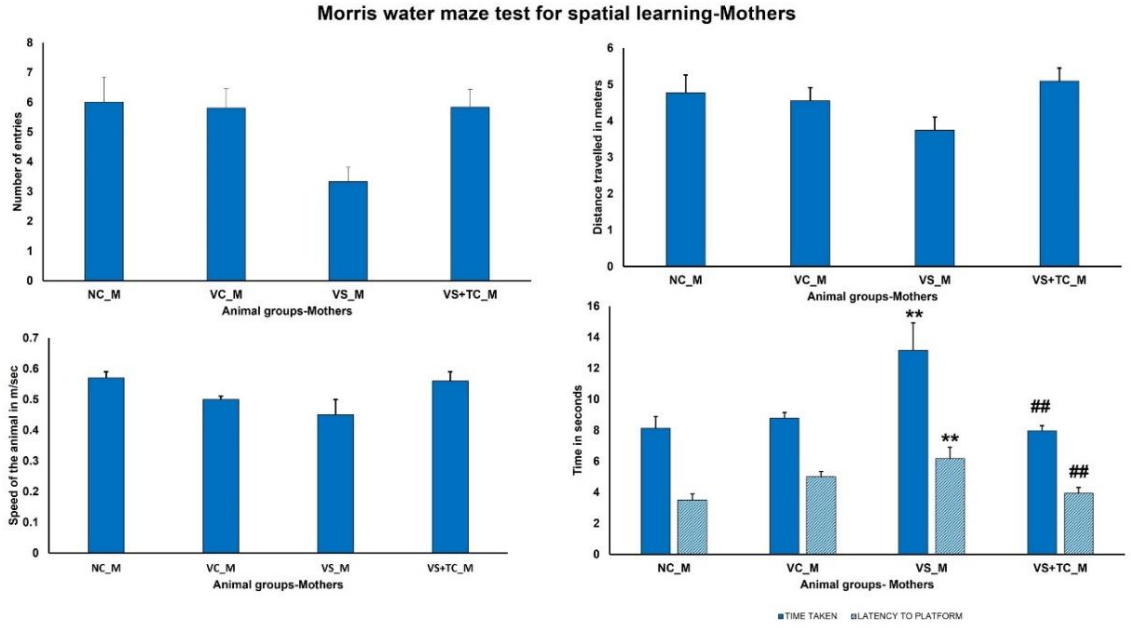


Figure 1: Morris water maze performance (Mean ± SEM) of mothers.

2.3 Vibration stress Induction:

The machine which induces the vibration produces 72 oscillations/minute. The dams were induced with vibration stress by vibrating them on a horizontal oscillatory tray of the machine from the 7 to 16 days of their gestation, every day for 3hrs. The duration of vibration stress was optimized during our preliminary studies.

2.4 *Tinospora cordifolia* administration

The herbal extract from stem of TC was supplied by the Himalaya wellness company. The shelf life of this extract was 2 years. 6mg of TC extract /kg was fed to dams belonging to treatment groups with 5% saline, every day prior to the vibration stress. The pups belonging to postnatal treatment groups were also given 6mg/kg of TC extract along with 5% saline.

2.5 Morris water maze test to assess spatial learning behavior:

After the weaning period was over the dams were subjected to Morri's water maze test. The neonatal groups were subjected to the same task when they were about 6 weeks old. The apparatus consists of a water pool of 180cm in diameter and 75cm in depth. The tank was divided into four quadrants by designating four places on its rim as N, S, E, and W. A platform of 4 x 4 inches was positioned deep to the water's surface in one of the quadrants which is identified as the target quadrant. Two images were placed on the walls to give the rats extra-maze clues so that they could learn a spatial map technique. Throughout the duration of the experiment, the cues' positions remained constant.

Acquisition phase

The rats received eleven sessions of training in the water maze over six days in a row, with two sessions a day except day 1. On day 1, only one session of training was conducted. Four trials were conducted in each session. Different spots within the tank were always used to start the trials. The platform's placement in the target quadrant

stayed the same for each rat, but the rats were placed into the pool at different quadrants on different trials, making it impossible for the rat to predict the platform's location. 3 minutes were given to the rats to find the platform. If the rat was successful in finding the platform, it was allowed to stay there for 10 seconds. The latency, or time it took to get to the hidden platform, was noted during each training session. The rat was directed to the target and allowed to be there for ten seconds if it couldn't locate it in three minutes. Following every experiment, the rats were taken out, patted dry with a dry cloth, and returned to the cages.

Probe phase

Each rat was put through a 30-second probing trial without a hidden platform the day after the last training session ended. The time taken by the rat to reach the target, the distance travelled to reach the target, and the time spent in the target quadrant are noted. Every incident was captured on video using a camera and the images were used to analyze the data. Memory impairment was indicated by a greater delay in reaching the target, and a shorter duration spent in target quadrant.

2.6 Golgi cox staining procedure

After completion of treatment, the dams and pups were anesthetized and sacrificed. The hippocampi were processed for "Golgi cox" staining[7]. "Golgi cox" solution for impregnation was prepared by mixing equivolume of potassium dichromate, mercuric chloride and potassium chromate and was kept in the dark for 2 days. Freshly dissected brains were rinsed in double distilled water and immersed in this solution for a week. Following this the brain tissues were kept immersed in 30% sucrose for about 8 hours. Then tissue sections of about 120µ thickness were taken. The sections were then treated with 75% ammonia solution and 1% sodium thiosulfate for about 10 minutes under darkness and treated with ascending grades of alcohol for dehydration. Tissue slides were mounted, and photographs were captured under the microscope with 20X magnification. Hippocampal CA3 neuronal dendritic arborization (intersections and branching points) were quantified using Sholl's circle method[8 9] .

2.7 Statistical analysis

Analysis of data was done with SPSS (version 20) and results were represented as Mean±SEM. Different groups were compared using ANOVA followed by "Bonferroni's" post hoc test with $p<0.05$ as a determinant of significance.

3. RESULTS

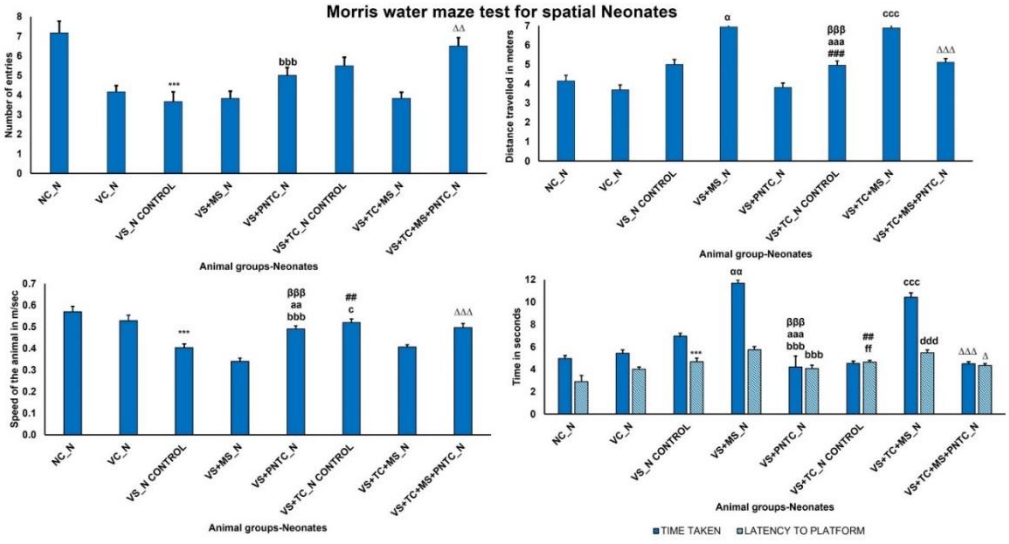
3.1 Morri's water maze for assessing the spatial learning

3.1A. Results of spatial learning task of mothers

Figure 1 shows Morri's water maze test for spatial learning tasks of mothers. The data suggests that TC treated mothers showed significantly improved performance compared to vibration stressed mothers during the spatial learning tasks. The longer time taken by the vibration stressed mothers to enter the target quadrant and reach the platform shows impaired spatial memory.

3.1B. Results of spatial learning task of neonates

The test parameters as shown in Figure 2 during Morri's water maze test reveals decreased number of entries and mean distance (in meters) travelled by animal to reach the target quadrant in the neonates subjected to prenatal vibration stress and postnatal maternal separation. Both prenatal and postnatal TC treated neonates took significantly ($p<0.001$) less time (in seconds) to reach the platform compared to stressed groups. When TC treated, pups were compared with prenatally vibration stressed and maternal separation groups, latency to reach the platform in the target quadrant was reduced, implying that TC treatment enhanced the spatial learning behaviour in these groups. TC treated neonates moved faster to reach the platform compared to stressed groups. Furthermore, these findings also show that, in comparison to the prenatal TC treated animals, the postnatal TC treatment group showed a higher degree of improvement in spatial learning behavior.



NC- N vs VS-N *** p<0.001; VS-N Control v/s VS+TC-N Control ## p<0.01, ### p<0.001; VS-N Control v/s VS+MS-N α p<0.05; VS+TC-N Control v/s VS+PNTC-N βββ p<0.001; VS+PNTC-N vs VS+TC+MS+TC-N Δ p<0.05; VS-N Control v/s VS+PNTC-N aa p<0.01, aaa p<0.001; NC-N v/s VS+PNTC-N bb p<0.01, bbb p<0.001; VS+MS-N v/s VS+TC+MS-N c p<0.05, ccc p<0.001; ddd NC-N vs VS+TC+MS-N p<0.001; ff NC-N vs VS+TC-N Control p<0.01 (Bonferroni's post hoc test)

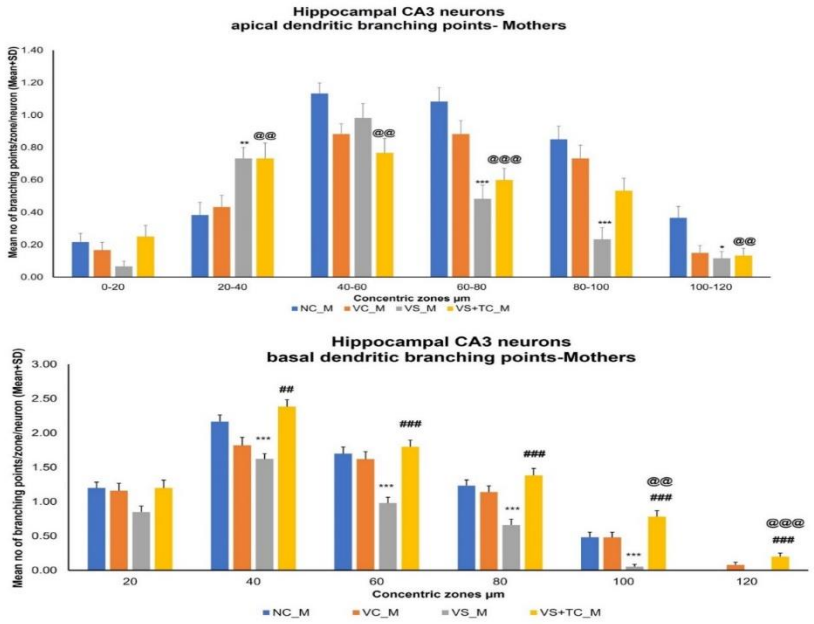
Figure 2: Morris water maze performance (Mean ± SEM) of neonates.

3.2. Dendritic morphology of CA3 neurons of hippocampus

3.2A. Quantification of neuronal dendritic arborization in mother rats

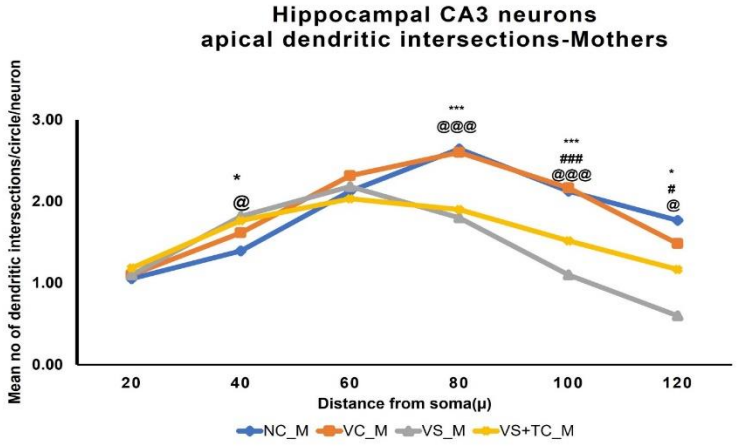
Hippocampal CA3 neuronal apical dendritic branching points were significantly increased from 20µ-60µ concentric zones from the soma whereas increase in basal dendritic branching points was observed from 20µ-100µ concentric zones from the soma in mother rats which underwent TC treatment (VS+TC-M) compared to vibration stress (VS-M) group. The reduced dendritic arborization in the VS -M group shows aberrant long-term potentiation (LTP) of synaptic activity in CA3 neurons caused by vibration stress, as reflected in this group's poor performance in spatial learning tests (Figure 3).

The VS -M group had a lower number of apical dendritic intersections (60µ-100µ concentric zones), implying that vibration stress influences dendritic arborization of hippocampal CA3 neurons. However, in TC-treated mothers, a considerable increase in apical dendritic intersections at 60µ-120µ concentric zones was seen, indicating that TC had neuroprotective characteristics (Figure 4).



NC- M v/s VS- M *p<0.05, **p<0.01, ***p<0.001; VS- M v/s VS+TC- M #p<0.05, ###p<0.001; NC- M v/s VS+TC- M @@p<0.01, @@@ p<0.001 (Bonferroni's post hoc test).

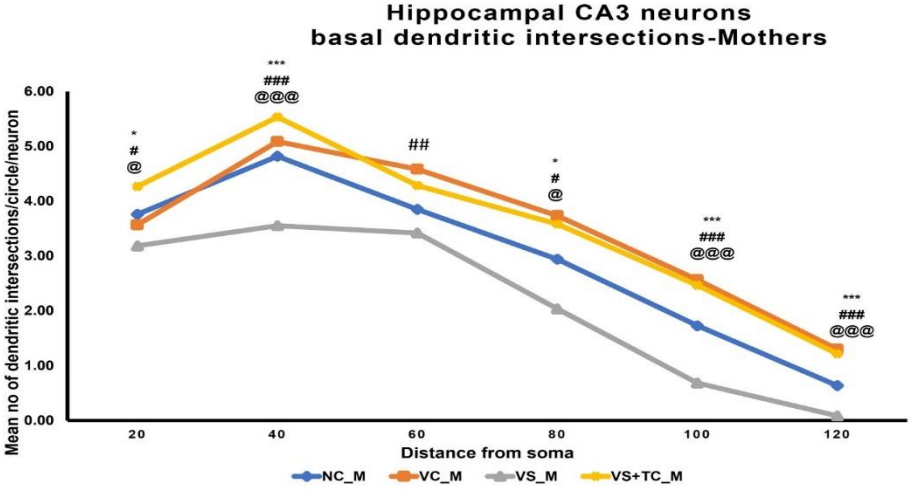
Figure 3: Dendritic branching points (Mean ± SEM) of CA3 neurons of hippocampus of mothers.



NC-M v/s VS-M *p<0.05, ***p<0.001; VS-M v/s VS+TC-M ###p<0.001; NC-M v/s VS+TC-M @p<0.05, @@@ p<0.001 (Bonferroni's post hoc test).

Figure 4: Dendritic intersections of "hippocampal CA3" neurons of mothers at various concentric circles.

Basal dendritic intersections of "hippocampal CA3" neurons were higher in concentric circles (20µ-60µ) close to the soma compared to that of apical intersections (Figure 5). (Figure 6)



NC- M v/s VS-M *p<0.05, ***p<0.001; VS- M v/s VS+TC-M #p<0.05, ## p<0.01, ### p<0.001; NC-M v/s VS+TC-M @p<0.05, @@@ p<0.001(Bonferroni's post hoc test).

Figure 5: Dendritic intersections of "hippocampal CA3" neurons of mothers at various concentric circles.

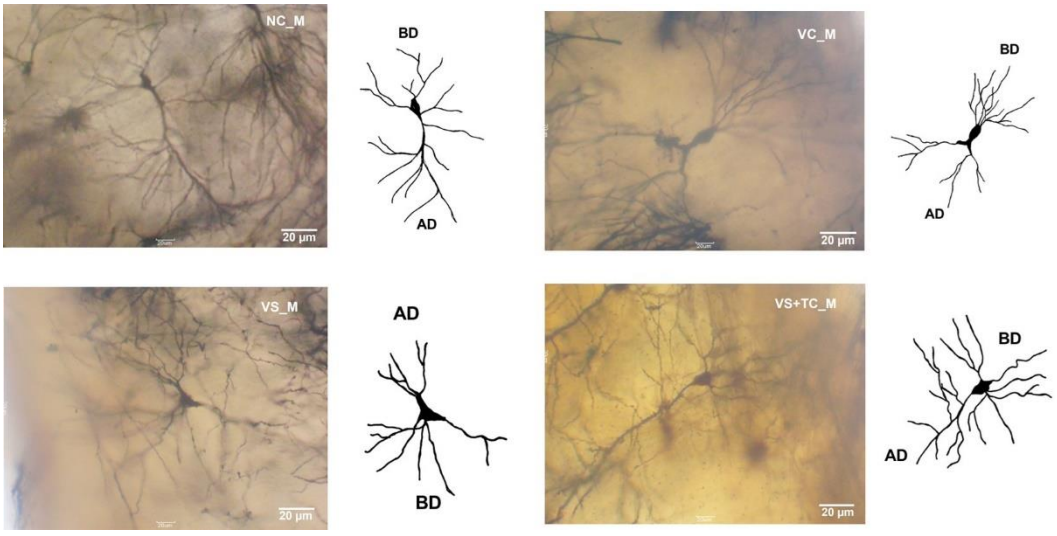
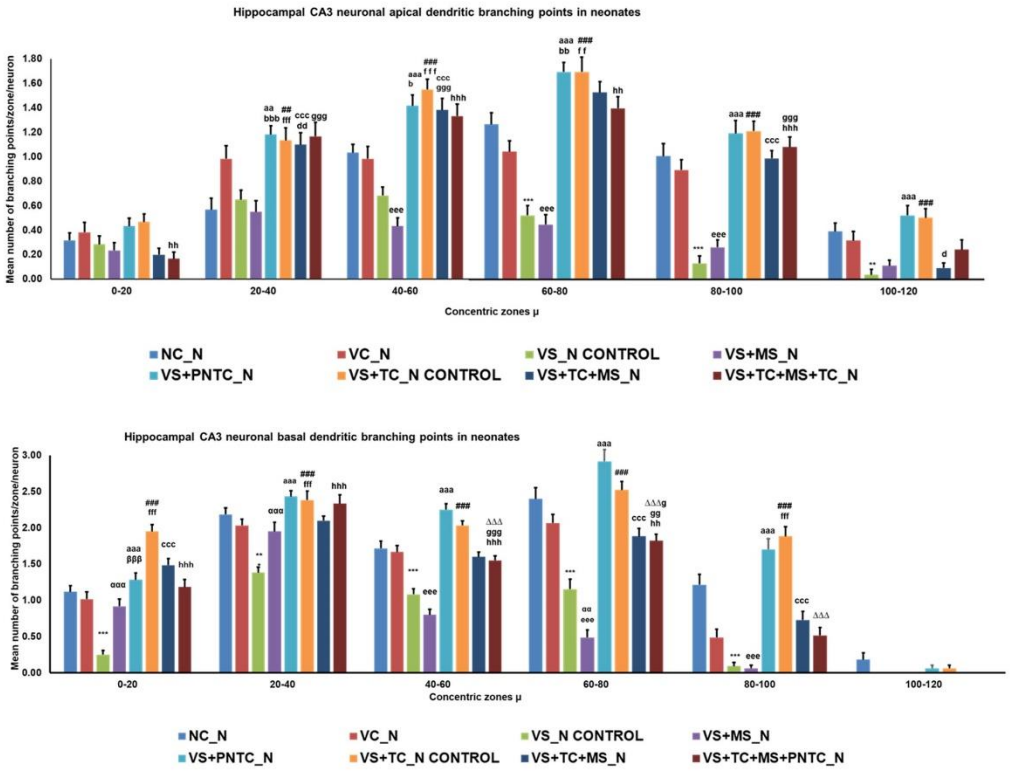


Figure 6: Sample photomicrographs of hippocampal CA3 neurons and tracings of those neurons. AD-Apical dendrites, BD-Basal dendrites

3.2B. Quantification of neuronal dendritic arborization in neonates

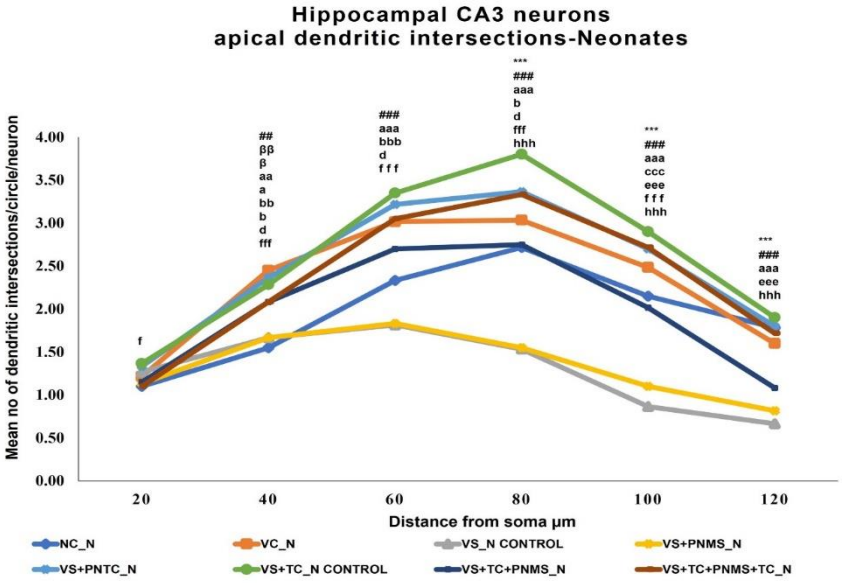
Prenatally stressed (VS-N Control) pups and pups which underwent maternal separation (VS+MS-N) had fewer apical as well as basal dendritic branching points compared to that of control and prenatal and postnatal TC treated pups. The apical dendritic branching points of postnatally TC treated pups (VS+PNTC N) increased significantly from 20µ-80µ concentric zones, although the rise in basal dendritic branching points was noticeable at the outer zones of Sholl's circle (40µ-100µ) (Figure 7)

Increase in apical dendritic intersections was also significant at 60µ to 100µ concentric circles from the soma of hippocampus CA3 neurons (Figure 8). Basal dendritic intersections were higher at inner zone ranging from 20µ to 80µ from the soma. (Figure 9). (Figure 10)



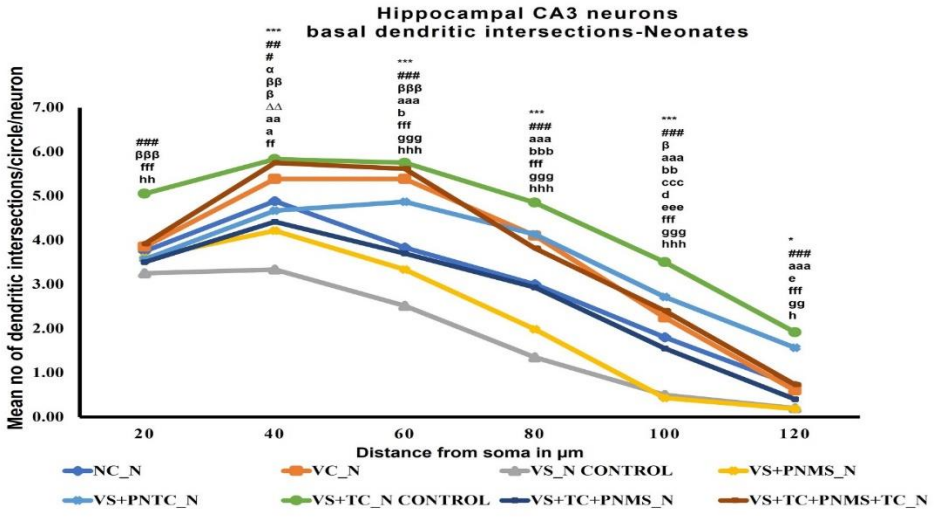
NC-N v/s VS-N Control, *p<0.05; VS-N Control v/s VS+TC-N Control, ##p<0.01; VS-N Control v/s VS+MS-N, aa p<0.01, aaa p<0.001; NC-N Control v/s VS+PNTC-N, bbb p<0.001; VS+MS-N v/s VS+TC+MS-N, ccc p<0.001; NC-N v/s VS+TC+MS-N, dd p<0.01; NC-N v/s VS+TC-N Control, f f f p<0.001; VS+MS-N v/s VS+TC+MS+PNTC-N, ggg p<0.001; VS+-N Control v/s VS+TC+MS+PNTC-N, hhh p<0.001 (Bonferroni's post hoc test).

Figure 7: Branching points of dendritic (Mean ± SEM) of CA3 neurons of hippocampus of neonates.



NC-N v/s VS-N Control, ***p<0.001; VS-N Control v/s VS+TC-N Control, ##p<0.01, ### p<0.001; VS+TC-N Control v/s VS+PNTC-N, βββ p<0.001; VS-N Control v/s VS+PNTC-N, aaa p<0.001; NC-N Control v/s VS+PNTC-N, b p<0.05, bbb p<0.001; VS+MS-N v/s VS+TC+MS-N, ccc p<0.001; NC-N v/s VS+TC+MS-N, d p<0.05; NC-N v/s VS+MS-N, eee p<0.001; NC- N v/s VS+TC-N Control, f p<0.05, fff p<0.001; VS+N Control v/s VS+TC+MS+PNTC-N, hhh p<0.001 (Bonferroni's post hoc test).

Figure 8: Dendritic intersections of "hippocampal CA3" neurons of neonates at various concentric circles.



NC- N v/s VS-N Control, *p<0.05, *** p<0.001; VS-N Control v/s VS+TC-N Control, ## p<0.01, ### p<0.001; VS-N Control v/s VS+MS-N α p<0.05; VS+TC-N Control v/s VS+PNTC-N, β p<0.05, βββ p<0.001; VS-N Control v/s VS+PNTC-N, aaa p<0.001; NC-N Control v/s VS+PNTC-N, b p<0.05, bb p<0.01, bbb p<0.001; VS+MS- N v/s VS+TC+MS-N, ccc p<0.001; NC-N v/s VS+TC+MS-N, d p<0.05; NC-N v/s VS+MS-N, e p<0.05, eee p<0.001 ; NC-N v/s VS+TC-N Control, ff p<0.01, fff p<0.001; VS+MS-N v/s VS+TC+MS+PNTC-N gg p<0.01, ggg p<0.001; VS+-N Control v/s VS+TC+MS+PNTC-N, h p<0.05, hh p<0.01, hhh p<0.001 (Bonferroni's post hoc test).

Figure 9: Dendritic intersections of "hippocampal CA3" neurons of neonates at various concentric circles.

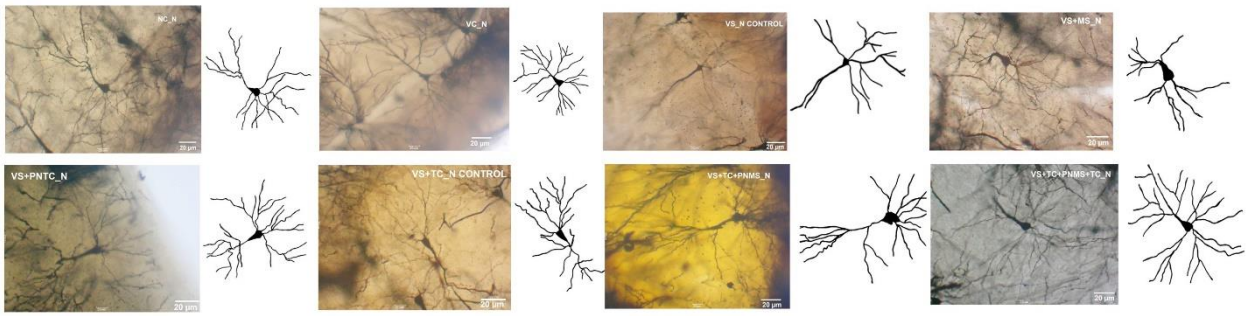


Figure 10: Sample photomicrographs hippocampal CA3 neurons and tracings of those neurons. AD-Apical dendrites, BD- Basal dendrites.

3.3. Analysis of correlation between neuronal dendritic morphology and spatial learning behaviour

3.3A. Correlation between CA3 dendritic branching points and number of entries to target quadrant during Morri's water maze test of mothers

The graphs demonstrate a linear relationship between the control group and the vibration stressed mothers. When compared to vibration stressed mothers, the mean value of number of entries to the target quadrant was higher in stress and TC extract treated mothers. Even though the VS+TC-M group had higher number of branching points of dendrites, the pruned dendrites in the VS-M group permitted these vibration stressed mothers to complete their normal baseline behavioral functions during Morris water maze task (Figure 11).

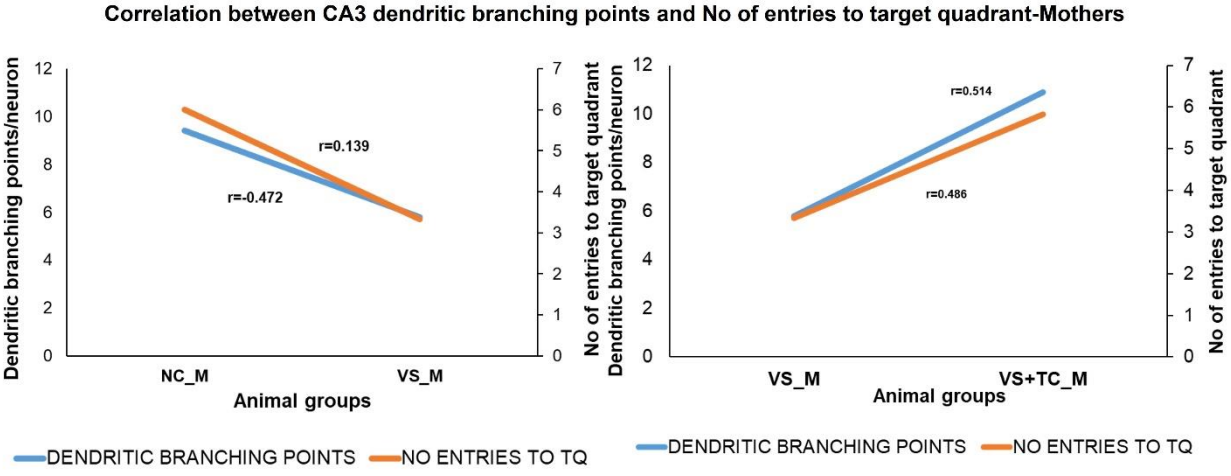


Figure 11: Correlation between branching points "hippocampal CA3" neuronal dendrites and number of entries to the target zone during Morri's water maze test for spatial learning in mothers. r= Pearson's correlation coefficient.

3.3B. Correlation between CA3 dendritic branching points and number of entries to target quadrant during Morri's water maze test of neonates

A linear correlation was observed between the NC-N and VS-N control neonatal groups. Increase in hippocampal dendritic branching points were observed in neonates which underwent vibration stress and stress induced by maternal separation along with prenatal as well

as postnatal TC extract treatment. However, both VS+TC-N control and VS+PNTC-N groups showed an increase in the branching points of dendrites, but there was no significant improvement in their performance during the spatial learning task. (Figure 12)

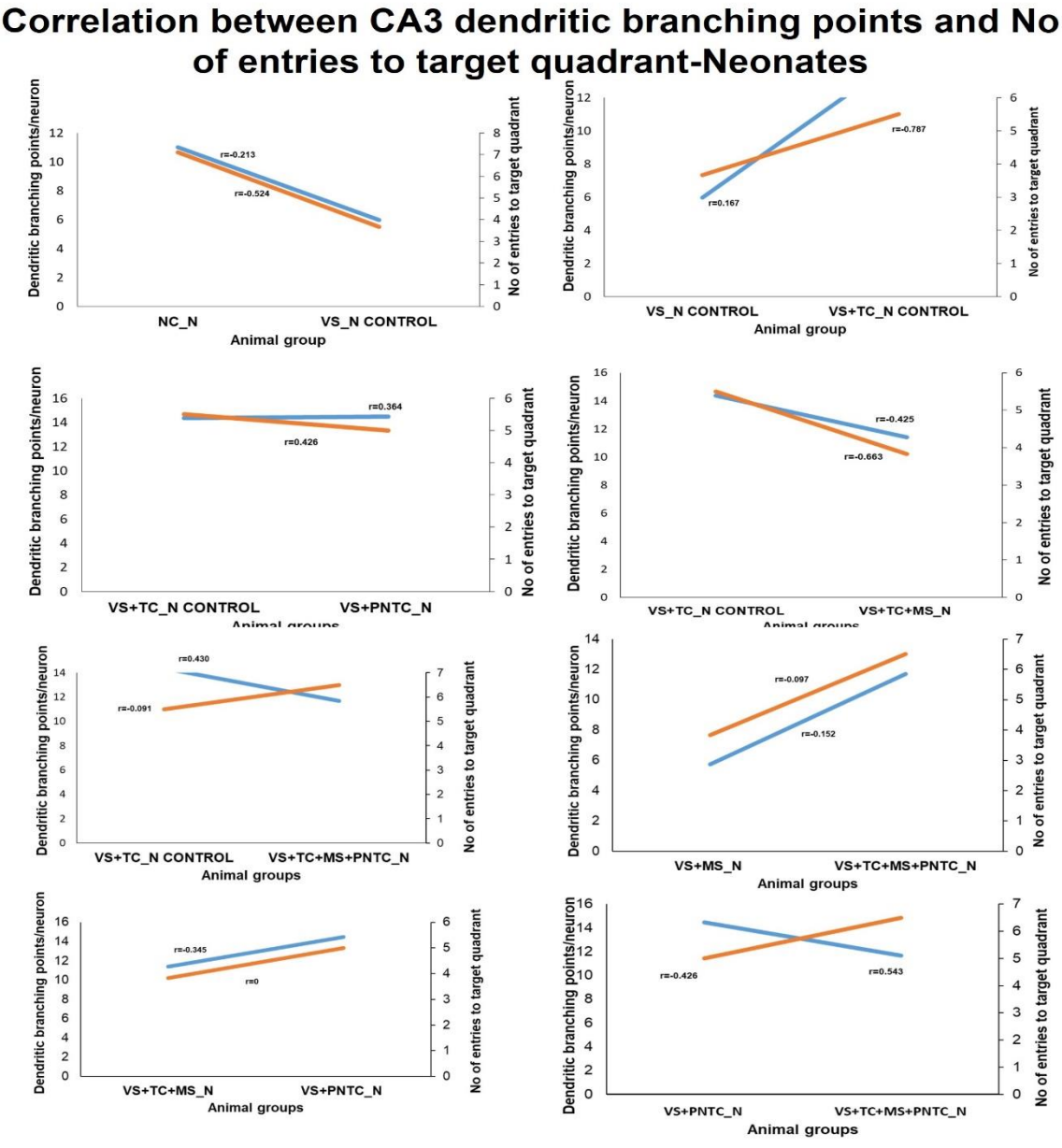


Figure 12: Correlation between hippocampal CA3 dendritic branching points and number of entries to target quadrant during Morri's water maze test for spatial learning in neonates. r= Pearson's correlation coefficient.

4. DISCUSSION

The current study's objectives were to analyze the i) the effect of prenatal vibration stress and the stress induced by postnatal maternal separation on structural plasticity observed in hippocampal CA3 region ii) the treatment with TC attenuating the detrimental effect of prenatal vibration stress and stress induced by postnatal maternal separation on CA3 neurons of hippocampus; an area involved in spatial learning behaviour iii) the effect of TC treatment in improving hippocampal dependent spatial learning in animals subjected to prenatal vibration stress and in animals stressed by postnatal maternal separation

Prenatal stress induces series of disorders affecting the behaviour and morphological changes in hippocampal formation[21011]. Early life stress due to maternal separation impairs cognitive performances and lead to several psychotic disorders[1213]. These stressors increase the free radicals which result in neuronal damage in these animals. Many different stress models have been tested in animals and humans to see how stress affects learning and memory [1415]. With these considerations, a novel model of stress induction was created to mimic the vibration stress experienced by a pregnant woman, which will influence the brain development of infants [16 17].

Tinospora cordifolia contains anti-oxidant, memory-enhancing, and immune-modulator properties [514]. According to Agarwal et al., both aqueous and ethanol extracts of TC improves cognition in cyclosporine-induced memory loss in rats [1516]. Butanol extract of TC has been known to alleviate the neurobehavioral impairments by modulating the hippocampal dendritic growth, synaptic plasticity, learning and memory formation in sleep deprived rats [17]. In our study, latency period to reach the hidden platform during Morri's water maze test was significantly longer in vibration stressed dams and neonates. Treatment with *Tinospora cordifolia* enhanced the hippocampus involved spatial learning in both dams and neonates. In comparison to stressed groups, pups treated with both prenatal and postnatal *Tinospora cordifolia* performed better and remembered the visual signals to reach the hidden platform. TC improved the memory by reducing the adverse effect of prenatally induced stress as well as stress induced by postnatal maternal separation-induced cognitive deficits.

Role of hippocampus in learning and memory pathways in human and rodent is well documented in the literature. Long-term potentiation of hippocampus dendritic spines, which carry excitatory synapses, are involved in the physiological process of memory formation[18]. Early life stress such as prenatal stress and postnatal maternal separation significantly influences the brain development and results in enhanced secretion of maternal corticosterone hormones leading to disturbance of hypothalamic-pituitary-adrenal axis (HPA axis) [1,19, 20]. Glucocorticoids-dependent reduction in the hippocampal volume, arborization and neurogenesis observed in major depressive disorders[21].

Stress mediated neuronal plasticity in the hippocampal CA3 region is suggested by the decrease in branching points of dendrites and number of intersections in stressed dams and neonates in our study. Neonates which underwent prenatal vibration stress and postnatally separated from mothers showed a significant loss in dendritic spines, which was attenuated by both prenatal and postnatal TC therapy. Postnatal TC extract administered neonates (VS+PNTC N) had considerably more apical and basal dendritic branching points than prenatal TC extract treated groups (VS+TC N). Thus, proving neuroprotective potential of TC by mitigating the structural destruction caused by prenatal vibration stress as well as stress induced by postnatal separation of animals from their mothers. As we can see in results, there was a significant increase in basal dendritic intersections in TC extract treated mothers and pups. This indicates a possible increased synaptic activity of hippocampal CA3 neuronal basal dendrites and mossy fibers of granule cells in the dentate gyrus, which may have further alleviated stress-induced hippocampal plasticity in these animals.

Butanol extract of TC treatment improved novel object

recognition performance and decreased the cellular stress and control cell apoptosis in the hippocampal region and also in piriform cortex of sleep deprived animals [17]. Similar *medhya* drugs like *Centalla asiatica*, *Withamnia somnifera, Bacopa monnieri* and *Ocimum sanctum* extracts have known to enhance the hippocampal CA3 neuronal density in turn fascilitating the spatial learning and memory retention in rodents[21,22,23,24,25, 26, 27, 28]. Kusindarta has found that *Ocimum sanctum* treatment elevates the acetyl choline neurotransmitter concentration and increase in pyramidal neurons density of hippocampal CA1 and CA3 regions [29]. In the present study also we were able to establish a correlation between the hippocampal dendritic morphology and spatial learning behavior of rats which were stressed and the stressed rats which were treated with TC extract.

A strong "positive linear correlation" was observed between the spatial learning performance and branching points of hippocampal CA3 neuronal dendrites in the mothers and also in rats of neonatal groups. This suggests that prenatal vibration stress as well as stress resulting from the postnatal separation of pups from their mothers affects spatial learning in these animals. The significant increase in the branching points of hippocampal CA3 neuronal dendrites in both prenatal and postnatal TC treated pups showed improved spatial learning behavioral outcomes. However, VS+TC+MS+PNTC-N group which received both prenatal and postnatal TC treatment prior to prenatal vibration stress as well as stress resulting from the postnatal separation of pups from their mothers showed increased number branching points of dendrites of hippocampal CA3 neurons along with improved performance in spatial learning behavioral tests.

5.CONCLUSIONS

The early life deleterious factors such as prenatal stress and maternal separation have profound detrimental effects on CA3 neurons of hippocampus and spatial learning. Improved learning and hippocampal CA 3 neuronal dendritic arborization in TC treated rats suggests the neuroprotective potential of *Tinospora cordifolia* extract. The improved behavioral performance and better structural morphology in postnatal TC extract treated neonates may be attributed to the limited ability of active compounds of TC to cross the placental barrier, which is yet to be confirmed. We also hope that TC could be used as a household remedy against daily stressors and the stress during pregnancy in women.

Author Contributions:

Conceptualization: Dr. Mohandas Rao KG, Ms. Ashwini LS

Data curation: Ms. Ashwini LS

Formal analysis: Ms. Ashwini LS

Funding acquisition: NA

Investigation: Ms. Ashwini LS

Methodology: Dr. Mohandas Rao KG, Ms. Ashwini LS, Dr. Guruprasad Rao, Dr. Padmanabha Udupa, Dr. Somashekar Shetty B, Dr. Chinmay Ajit Suryavanshi

Project administration: Dr. Mohandas Rao KG, Dr. Kiranmai Rai

Resources: Guruprasad Rao, Dr. Padmanabha Udupa, Dr. Somashekar Shetty B, Dr. Chinmay Ajit Suryavanshi

Software: Ms. Ashwini LS, Dr. Chinmay Ajit Suryavanshi

Supervision: Dr. Mohandas Rao KG, Dr. Kiranmai Rai, Dr. Somashekar Shetty

Validation: Dr. Mohandas Rao KG, Dr. Kiranmai Rai, Dr. Somashekar, Shetty Guruprasad Rao, Dr. Padmanabha Udupa

Visualization: Dr. Mohandas Rao KG, Ms. Ashwini LS

Writing–original draft: Ms. Ashwini LS

Writing–review & editing: Dr. Mohandas Rao KG, Ms. Ashwini LS

Declaration of Conflicting Interests: None

Funding: None

Ethical consideration:

The protocol was approved by Institutional Animal Ethical Committee, Manipal Academy of Higher Education, Manipal, India (Approval letter Reference No. IAEC/KMC/56/2015).

REFERENCES

1. Abrous N, Koehl M, Lemaire V, Le Moal M. Prenatal stress produces cognitive impairment with an inhibition of neurogenesis in the hippocampus. *Medecine/Sciences*. 2001;17(1):119-121. doi:10.4267/10608/1800
2. National Scientific Council on the Developing Child. Excessive Stress Disrupts the Architecture of the Developing Brain. Published online 2014.
3. Preety D, Pooja, Deepak S, Mradu B, Nalini S. Evaluation of in vitro cytoprotective and antioxidant effects of Tinospora cordifolia in cultured HepG2 cells. *J Herb Med*. 2022;31:100529. doi:10.1016/J.HERMED.2021.100529
4. Arunachalam K, Yang X, San TT. Tinospora cordifolia (Willd.) Miers: Protection mechanisms and strategies against oxidative stress-related diseases. *J Ethnopharmacol*. 2022;283:114540. doi:10.1016/J.JEP.2021.114540
5. Sharma U, Bala M, Kumar N, Singh B, Munshi RK, Bhalerao S. Immunomodulatory active compounds from Tinospora cordifolia. *J Ethnopharmacol*. 2012;141(3):918-926. doi:10.1016/j.jep.2012.03.027
6. Chi S, She G, Han D, Wang W, Liu Z, Liu B. Genus Tinospora: Ethnopharmacology, Phytochemistry, and Pharmacology. *Evidence-based Complement Altern Med*. 2016;2016. doi:10.1155/2016/9232593
7. Gitanjali Das et al. The Golgi-Cox method. 2013;(February). doi:10.1007/978-1-62703-444-9
8. Rao KGM, Rao SM, Rao SG. Centella asiatica (L .) Leaf Extract Treatment During the Growth Spurt Period Enhances Hippocampal CA3 Neuronal Dendritic Arborization in Rats. 2006;3(June):349-357. doi:10.1093/ecam/nel024
9. O'Neill KM, Akum BF, Dhawan ST, Kwon M, Langhammer CG, Firestein BL. Assessing effects on dendritic arborization using novel Sholl analyses. *Front Cell Neurosci*. 2015;9(JULY):1-14. doi:10.3389/fncel.2015.00285
10. Aleksandrov AA, Polyakova ON, Batuev AS. The Effects of Prenatal Stress on Learning in Rats in a Morris Maze. *Neurosci Behav Physiol 2001 311*. 2001;31(1):71-74. doi:10.1023/A:1026682415860
11. Svanidze IK, Museridze DP, Didimova E V, Sanikidze T V, Gegenava LG, Gvinadze NN. *Disordered Neurogenesis in Cortical and Subcortical Limbic System Structures in the Rat Brain in Fetal Alcohol Syndrome*. Vol 43.; 2013.
12. Reincke SAJ, Hanganu-opatz IL. Early-life stress impairs recognition memory and perturbs the functional maturation of prefrontal- hippocampal-perirhinal networks. *Nat Publ Gr*. 2017;(February):1-16. doi:10.1038/srep42042
13. Banqueri M, Méndez M, Arias JL. Physiology & Behavior Spatial memory-related brain activity in normally reared and di ff erent maternal separation models in rats. 2017;181(September):80-82.
14. Saha S, Ghosh S, Marg S. Tinospora cordifolia : One plant, many roles. 2012;31(4):151-159. doi:10.4103/0257-7941.107344
15. Agarwal A, Malini S, Bairy KL, Rao MS. Effect of Tinospora cordifolia on learning and memory in normal and memory deficit rats. *Indian J Pharmacol*. 2002;34(5):339-349.
16. Singh D, Chaudhuri PK. Chemistry and Pharmacology of Tinospora cordifolia. Published online 2017:33-41. doi:10.1177/1934578X1701200240
17. Bajaj P, Singh H, Kalotra S, Kaur G. Butanol Extract of Tinospora cordifolia Alleviates Acute Sleep Deprivation-Induced Impairments in Cognitive Functions and Neuromuscular Coordination in Middle-Aged Female Rats. *NeuroMolecular Med*. 2021;(0123456789). doi:10.1007/s12017-021-08683-x

18. Chen Y, Rex CS, Rice CJ, Dubé CM, Gall CM, Lynch G. Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling. 2010;107(29):13123-13128. doi:10.1073/pnas.1003825107
19. Warner-Schmidt JL, Duman RS. Hippocampal neurogenesis: Opposing effects of stress and antidepressant treatment. *Hippocampus*. 2006;16(3):239-249. doi:10.1002/hipo.20156
20. Dranovsky A, Hen R. Hippocampal Neurogenesis: Regulation by Stress and Antidepressants. *Biol Psychiatry*. 2006;59(12):1136-1143. doi:10.1016/j.biopsych.2006.03.082
21. Rao MKG, Rao MS, Rao GS. Treatment with Centalla asiatica (Linn) fresh leaf extract enhances learning ability and memory retention power in rats. *Neurosciences*. 2007;12(3):236-241.
22. Jo KJ, Nam GH, Park YS, et al. Evaluation of Stress-related Behavioral and Biological Activity of Ocimum sanctum Extract in Rats. *Biotechnol Bioprocess Eng*. 2020;25(2):170-180. doi:10.1007/s12257-019-0365-2
23. Husain GM, Mishra D, Singh PN, Rao C V, Kumar V. Ethnopharmacological review of native traditional medicinal plants for brain disorders. *Pharmacogn Rev*. 2007;1(1):19-29. http://www.phcogrev.com
24. Campus R. Cognitive Enhancement Effects of Bacopa monnieri (Brahmi) on Novel Object Recognition and Neuronal Density in the Prefrontal Cortex , Striatum and Hippocampus in Sub-Chronic Phencyclidine Administration Rat Model of Schizophrenia. 2015;98(6):56-63.
25. Manchanda S, Mishra R, Singh R, Kaur T, Kaur G. Aqueous Leaf Extract of Withania somnifera as a Potential Neuroprotective Agent in Sleep-deprived Rats: a Mechanistic Study. *Mol Neurobiol*. 2017;54(4):3050-3061. doi:10.1007/s12035-016-9883-5
26. Jagadeeshwar, Kolguri & Rupaka, Subhakar & Rajasekhar Reddy, Alavala & Gsn, Koteswara Rao & Kulandaivelu, Umasankar. (2023). Investigation of Nootropic and Neuroprotective Activity of Myristica malabarica Bark Extracts on STZ induced Cognitive Impairment in Experimental Animals. *Jordan Journal of Pharmaceutical Sciences*. 171-183. 10.35516/jjps.v16i2.1318.
27. Ha, V. T., & Le, N. T. (2022). Extraction of anthocyanins from Clitoria ternatea L. petals in Vietnam and determination of its antioxidant and antimicrobial activities. *Jordan Journal of Pharmaceutical Sciences*, 15(2), 145–157. https://doi.org/10.35516/jjps.v15i2.302
28. Huynh, D. T. M., T. Le, M.-N., Tran, V. D., Tran, V.-H., & Pham, D. T. (2023). Native Medicinal Plants (Moringa oleifera Lam, Brucea javanica (L.) Merr., Eclipta prostrata (L.), Callisia fragrans (Lindl.) Woodson, and Zingiber zerumbet (L.) Smith) in An Giang, Vietnam: A Preliminary Investigation for Rhabdomyosarcoma Treatments using in-vitro RD cell cytotoxicity test. *Jordan Journal of Pharmaceutical Sciences*, *16*(4), 830–841. https://doi.org/10.35516/jjps.v16i4.1365
29. Kusindarta DL, Wihadmadyatami H, Haryanto A. The analysis of hippocampus neuronal density (CA1 and CA3) after Ocimum sanctum ethanolic extract treatment on the young adulthood and middle age rat model. *Vet World*. 2018;11(2):135-140. doi:10.14202/vetworld.2018.135-140

الإمكانات العصبية الوقائية لـ Tinospora cordifolia في التخفيف من تلف الخلايا العصبية في الحُصين CA3 لدى فئران ويستار الحوامل وأطفالها حديثي الولادة المعرضين لإجهاد الاهتزاز قبل الولادة والانفصال الأمومي

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ملخص

الخلفية: يؤثر الإجهاد قبل الولادة سلبًا على الإدراك والسلوك والسمات النفسية والاجتماعية .يُعرف نبات تينوسبورا كورديفوليا (TC)بخصائصه المضادة للإجهاد والمعززة للإدراك .ومع ذلك، فإن فعاليته في مواجهة الإجهاد الناتج عن انفصال الأم واهتزازها غير موثقة جيدًا .هدفت الدراسة إلى تقييم الآثار العصبية الوقائية لنبات تينوسبورا كورديفوليا على الفئران حديثة الولادة التي تعرضت لإجهاد اهتزاز قبل الولادة، وكذلك الإجهاد الناتج عن انفصال الأم.

الطرق: تعرضت فئران ويستار الحوامل في المجموعة المعرضة للإجهاد لثلاث ساعات يوميًا من الإجهاد الاهتزازي، وذلك خلال الفترة من 7إلى 16يومًا من الحمل .أُعطيت مجموعة العلاج 6ملغم/كغم من مستخلص نبات تينوسبورا كورديفوليا قبل التعرض للإجهاد الاهتزازي .فُصل حديثو الولادة عن أمهاتهم وعولجوا بنبات تينوسبورا كورديفوليا بعد الولادة .في نهاية فترة العلاج، خضعت الفئران لمهمة تعلم مكاني .بعد ذلك، عولجت أدمغة الحيوانات لصبغة جولجي كوكس لدراسة التشجير العصبي .CA3

النتائج والاستنتاجات: أظهرت الأمهات المعالجات بمستخلص الكرياتينين تعلمًا مكانيًا أفضل بكثير من الأمهات اللاتي تعرضن لإجهاد الاهتزاز وحده .استغرق حديثو الولادة المعرضون لإجهاد ما قبل الولادة وقتًا أطول للعثور على الربع المستهدف، مما يشير إلى ضعف الذاكرة المكانية، والذي تحسن مع العلاج بمستخلص الكرياتينين .لوحظت زيادة في التفرع الشجيري في الخلايا العصبية CA3لدى كل من الأمهات المعالجات بمستخلص الكرياتينين وحديثي الولادة .يُحسّن مستخلص الكرياتينين التعلم المكاني لدى الفئران عن طريق تخفيف الضرر العصبي في الحصين CA3الناتج عن إجهاد الاهتزاز قبل الولادة وانفصال الأم.

الكلمات الدالة: إجهاد ما قبل الولادة، مستخلص عشبي، التعلم المكاني، الحُصين، تينوسبورا كورديفوليا.

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