Impact of noni juice on myelin components, neurotransmittory and behavioural status in rats exposed to immobilization stress

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Several reports indicate that psychological stress and depression are detrimental to health and that chronic stress implicates pathophysiology of mood and anxiety-related disorders, thus leading to several stress-related complications. The present study focuses on behavioural status and neurochemical variations in myelin of rat brain exposed to chronic immobilization stress and evaluates the effect of fruit juice of noni over changes in the myelin content of forebrain (FB) and midbrain (MB) regions of rats exposed to stress. Immobilization stress (IS) strategy was utilized to induce stress in Wistar rats. Myelin protein was isolated from the MB and FB regions of rats. Assessment of activities of membrane bound enzymes, levels of neurotransmitters (dopamine, serotonin and glutamate of FB and MB) and lipid profile of myelin in addition to behavioural analysis were carried out. The membrane-bound enzymes such as ATPases, 5'-nucleotidase and acetylcholinesterase exhibit significant decrease in their activities both in the FB and MB regions on stress induction, when compared to that of control. The neurotransmitters and lipid profile also show similar pattern among the experimental group. However, administration of noni juice (NJ) in IS-exposed rats can significantly alter the activities of myelin-associated enzymes and levels of neurotransmitters and lipid content of myelin (P < 0.05) along with improvement in the behavioural pattern when compared to those of IS rats. These preliminary results set targets for further authentication of the use of NJ against stress. This NJ could become one of the ideal supplements for management of stress-induced neurological disorders.

Keywords: Immobilization stress, myelin, noni juice, neurotransmitters, rats.

Stress is considered as an adverse reaction of the body which can perturb normal physiological equilibrium and undermine homeostasis. Stressful occasions elicit alterations in various organ systems, including gastrointestinal, cardiovascular and central nervous system. Stress is connected with post-traumatic stress conditions, major depression, schizophrenia and neurodegenerative diseases. Exposure to stress stimuli steers hyperactivation of hypothalamus–pituitary adrenal axis, behavioural changes, autonomic function and changes in adrenocorticotropin hormone as well as in corticosterone. Stress triggers structural abnormalities leading to loss of intact myelin and primary axonal degeneration, and impairment in the slow conduction velocity that results in secondary axonal degeneration. These restrained changes in myelin and white matter may progress to complicated ones as found in patients with psychiatric diseases, such as schizophrenia, who show wide differences in myelin and white matter.

Immobilization stress or restraint stress is a simple and suitable technique to induce both psychological (aggression, escape reaction) and physical (muscle work) stress, bringing about limited mobility and aggression. Several studies have reported that different kinds of stress are precipitated due to the generation of free radicals, and among the vital organs in human body, the central nervous system is more prone to oxidative damage. Recently, clinical utilization of indigenous medication for the treatment of different diseases is on demand. There seems to be a herbal renaissance throughout the globe. Recently, there is a global awareness in exploring the potential of products of herbal origin, rather than the synthetic compounds due to the possible side effects of the latter, that might aggravate the disease condition. Despite the fact that medicinal value of herbs was well-recognized over the centuries, the modern era has witnessed a surge in the use of synthetic compounds thereby masking the significance of natural ones. Nevertheless, reliance on synthetics is nowadays found to be overcome by an increasing trust about safety and security of natural products.

In the present study, we evaluate the effect of juice of noni fruit against stress insults. Noni juice (NJ) contains more than 160 identified chemicals; the major components include alkaloids, terpenoids, carotenoids, glycosides, rutin, vitamins A and C, and trisaccharide fatty acid ester. NJ is a potential antioxidant and prevents memory impairment as well as oxidative stress. The present study also explores the efficacy of NJ, which has been reported as safe for consumption, in reverting the
effects of immobilized stress in rat models with special reference to its effect on components of myelin.

**Experimental procedure**

**Animals**

Healthy male Wistar rats of 200–250 g weight were used in the study. Prior to initiation of the experimental procedure, the weighed animals were subjected to acclimatization by handling regularly for a period of 4 min, which was continued for seven successive days. Also, the rats were uniformly provided with food pellets and water and maintained in hygienic environment.

**Experimental groups:** There were four experimental groups with six rats in each. Group I, control (C); group II, immobilized stress (IS); group III, immobilized stress + treatment with NJ (5 ml/kg BMl), and group IV, control + NJ (CNJ) (5 ml/kg body wt).

**Immobilization stress:** Immobilization is a well-validated model to induce stress. In this study, the rats were exposed to 2 h immobilization stress for 21 days in transparent plastic apparatus specially designed for stress induction with air holes for aeration. After stress induction, the rats were put back in their respective home cage whereas the control rats were undisturbed in their cage. Immobilization stress procedure was carried out between 10.00 am and 06.00 pm. IS-induced rats were not given food and water during the procedure, whereas control and CNJ rats had unrestricted access to food and water. Food intake (calculated in terms of g/100 g body wt) and growth rate of the experimental animals were monitored regularly. Following IS induction procedure, the rats were co-treated with NJ orally for 21 days (5 ml/kg body wt). In addition, group-IV rats were subjected to NJ alone (5 ml/kg body wt). On the 22nd day, the rats were sacrificed by cervical decapitation; the brain was dissected out and washed with phosphate buffer saline. Midbrain (MB) and forebrain (FB) regions were used for further investigation.

**Behavioural assessment**

**Radial arm maze test:** Spatial memory test was done using eight-arm radial maze in which the experimental rats were put on dietary constraint instantaneously. After the last period of restraint, training sessions were carried out in order to avoid any biased reports on behavioural responses from the animals. The rats were allowed to go to the ends of the arms for a food reinforcement, as a result, they were rewarded with pellets. A visit to an arm was scored; however, if the rats went only three-fourths of the arm’s length, or if they entered the arm but did not eat the food, or re-entered into the arms that were previously visited, these were counted as mistakes. After the rats had made their choices, they were taken from the maze and put back in their respective home cages.

**Stride length test:** The hind paws of the animals were inked and footprints were made on a paper, which was a narrow runway of 1 m length and 7 cm breadth. Further, the track of each step was optimized in line. A sequence of at least five sequential steps was used for assessing the mean value for each stride length and it was calculated as the distance between the central pads of two successive prints on each side.

**Preparation of homogenate:** For this, 100 mg of FB and MB tissue was weighed and uniformly homogenized in an ice-cold aqueous solution of 0.20 M sucrose containing 1 mM EDTA, 1 mM β-mercaptoethanol (β-SH), and 0.25 mM phenylmethanesulphonyl fluoride (PMSF) (1 ml medium/0.100 g tissue) and the homogenate was used for the isolation of myelin sheath. This was done by density gradient centrifugation technique using the method of Norton and Poduslo.

**Isolation of myelin:** A 1 ml aliquot of homogenate was layered on 1 ml of 0.32 M sucrose and 3 ml of 0.78 M sucrose solution without shaking the tubes. Next, the tubes were centrifuged for 40 min in high-speed ultracentrifuge (106,000 g) at 4°C. Then 0.1 ml of diluted myelin was used for further assessment.

**Biochemical assays:** The total protein content from myelin was estimated by Lowry’s method. Further, the protein content of myelin was analysed by sodium dodecylsulphate polyacrylamide gel electrophoresis to ascertain the relative concentration of protein.

**Activity of membrane-bound enzyme:** The total sodium-potassium-ATPase activities of Mg2+-ATPase and Ca2+-ATPase and total 5′-nucleotidase activity were determined as reported earlier.

**Levels of neurotransmitter:** Total acetylcholinesterase activity, dopamine content and serotonin content were estimated as described earlier.

**Estimation of lipids:** The lipids from myelin membrane were isolated and myelin cholesterol level was assessed as described earlier. Phospholipids in myelin were determined by the method of Rouser et al.

**Thin layer chromatography:** Thin layer chromatography (TLC) was used for detection of total lipids, ceramide, cholesterol, glycolipids and phospholipids.

**Statistical analysis:** The experimental data were examined using one-way ANOVA utilizing the SPSS package.
Results of the experiments were expressed as mean ± SD. The levels of significance were set up at $P < 0.05$.

**Results and discussion**

The immobilization stress induces a psychological stress in the organism that is exposed to it. The type of stress response favours increase in anxiety and decrease in locomotor activity. In the present study, group-II rats (IS) showed a marked reduction ($P < 0.001$) in body weight (Figure 1) due to gradual reduction in the consumption of food and water (Figure 2). Rats treated with NJ alone showed no loss of appetite and consumption of food was similar to that of control. Interestingly, group-IV rats showed slight increase in consumption. On the other hand, the stressed rats treated with NJ (group III) presented normal body weight in comparison with those of control and stressed rats with normal BMI ($P < 0.001$).

These rats, though subjected to the same immobilization stress consumed food normally as those of control, owing to the administration of NJ though a marginal increase in the amount of food consumed by rats treated with NJ alone was observed.

When the experimental animals were subjected to behavioural tests, it was found during the maze test that the IS group of rats remained static in one of the arms, but those in group I (C), group III (NJ) and group IV (CNJ) were actively involved in the spatial test and found their reward (Figure 3). This showed that IS-induced group (group II) of rats might have been affected by regression of memory and also loss in exploring behaviour, which is normal in rodents. Thus, IS had weakened the working memory of group-II rats. This observation was similar to that of Victoria et al.\textsuperscript{27}, who observed that recurring stress exposure for 21 days reduced impact of preliminary learning in radial maze task. NJ showed significant ($P < 0.03$) improvement in reward finding by radial arm test (Figure 4) even when they were subjected

![Figure 1](image1.png)

**Figure 1.** Weight (g) of the experimental group of rats. Values are represented as mean ± SD (**$P < 0.01$, *$P < 0.05$). Comparison made: C versus all the other groups; IS versus NJ.

![Figure 2](image2.png)

**Figure 2.** Food (g) and water (ml) consumption (3 weeks) of the experimental group of rats. Values are represented as mean ± SD (**$P < 0.01$, *$P < 0.05$). Comparison made: C versus all the other groups; IS versus NJ.

![Figure 3](image3.png)

**Figure 3.** Spatial memory test obtained after behavioural assessment in the experimental group. Values are represented as mean ± SD (**$P < 0.01$, *$P < 0.05$). Comparison made: C versus all the other groups; IS versus NJ.

![Figure 4](image4.png)

**Figure 4.** Stride length (cm) test for assessing walking pattern of the animals under study. Values are represented as mean ± SD (**$P < 0.01$, *$P < 0.05$). Comparison made: C versus all the other groups; IS versus NJ.
to IS. Stride length test also showed improvement thus demonstrating the effect on stress. Group-IV rats showed no substantial variations in comparison with the control group. Therefore, administration of NJ to group-I and group-IV rats resulted in normal behavioural activity.

Myelin membrane is a compaction of glial cells around the axons with cytoplasmic extrusions and condensation of lipid membrane bilayers. The compactly packed myelin membrane supports the neuron with an insulating layer to avert exposure to extracellular milieu (ions and fluids). It is known that stress hampers the hypothalamic–pituitary–adrenal axis (HPA) and indirectly influences the integrity and fluidity of the myelin membrane. Figure 5 shows that myelin content of FB is comparatively less than that of MB. Significant increase (\( P < 0.05 \)) in myelin content was observed in IS-induced rats administered with NJ. Even though group-III rats (\( P < 0.05 \)) were subjected to stress induction similar to that of group-II rats, NJ administration resulted in increased myelin content thus showing that NJ has a protective impact on myelin integrity. Rats treated with NJ alone (group-IV) showed similar results when compared to that of control, thus highlighting that NJ does not exert any significant change in the content of myelin.

Myelin membrane is a lipid bilayer embedded with proteins, however, the protein content is comparatively less when compared with other biological membrane. There are few proteins present in myelin, such as MBP (myelin basic protein), PLP (proteolipid protein), oligodendrocytic protein, MAG (myelin associated glycoprotein), etc. The present study showed reduced content of total protein in rats subjected to IS (\( P < 0.001 \)) than those of control and group-III rats (Figure 6). The elevated protein content in group-III rats (\( P < 0.001 \)) suggests that NJ might combat the influence of stress. On the other hand, group-IV rats exhibited similar content of total protein as that of the control. The similar observation in group-I and group-IV rats ensures that NJ does not exert any adhere effect in control rats.

Figure 7 depicts the SDS electrophoretic pattern of myelin proteins isolated from all four groups. Protein expression in the FB region reveals less intense bands in all the groups, whereas the MB region shows predominant banding pattern. Group-II presents abundant number of less intense bands, thus revealing the loss of myelin proteins due to stress. Group-I exhibits dominant banding pattern which is comparable with the NJ group showing similar pattern of banding intensity. Groups I and IV exhibit similar banding pattern.

Sodium, potassium adenosine triphosphatases (Na, K-ATPases) are membrane-bound enzymes. They aid in transportation of \( \text{Na}^+ \) and \( \text{K}^+ \) ions, thus maintaining the ionic gradient which is essential for neuronal excitability and for regulating the neuronal cell volume. This activity was reported to be diminished in patients with bipolar disorder and depression. Kirshenbaum et al. observed that decreased activity of \( \text{Na}^+ \), \( \text{K}^-\)-ATPases can have an impact on the neuronal cell and on memory storage activity. There are also evidences supporting that immobilization stress could alter the functionality of \( \text{Na}^+ \), \( \text{K}^-\)-ATPase enzyme. In the present study, \( \text{Na}^+ \), \( \text{K}^-\)-ATPase showed reduction in its activity in IS group of rats (\( P < 0.001 \)) and the NJ-treated rats (\( P < 0.012 \)) showed a comparative increase in activity like those of the control group (Figure 8a). This observation is similar to previous studies in which the stress-induced models showed marked reduction in \( \text{Na}^+ \), \( \text{K}^-\)-ATPase activity. Thus, memory storage and neuronal activity which are dependent on \( \text{Na}^+ \), \( \text{K}^-\)-ATPase activity may have been affected on IS induction which, however, remains unaffected by NJ.

![Figure 5. Myelin content obtained after sucrose density centrifugation (mg/100 mg) of tissue from the brain of experimental group of rats. Values are represented as mean ± SD (**\( P < 0.01 \), *\( P < 0.05 \)). Comparison made: C versus all the other groups; IS versus NJ.](image)

![Figure 6. Total protein content of myelin from the experimental group of rats (\( \mu \text{g/mg of myelin} \)). Values are represented as mean ± SD (**\( P < 0.01 \), *\( P < 0.05 \)). Comparison made: C versus all the other groups; IS versus NJ.](image)
Ca\(^{2+}\)-ATPase is an ectoATPase vital for regulating the amount of Ca\(^{2+}\) within cells. Ca\(^{2+}\)-ATPase is localized in the paranodal regions of myelin fibres, signifying that there could be localized Ca flux in these fibres\(^2\). In this study, we observed an elevated activity of myelin membrane-bound Ca\(^{2+}\)-ATPase in IS rats on comparison with those of control and NJ groups (Figure 8b). This implies an impact on calcium flux and hence possible alteration in the calcium-dependent cell events. There was no significant change in Ca\(^{2+}\)-ATPase activity in both group-I and group-IV rats.

Magnesium plays a vital role in cellular processes by acting mainly as a cofactor in enzymatic reactions and transmembrane ion movements. Mg\(^{2+}\)-stimulated ATPase belongs to ectoATPases. In the brain, Mg\(^{2+}\)-stimulated ATPase activity has been found in neuronal and glial membranes, especially in neurotransmitter storage vesicles and synaptic plasma membranes. The function of Mg\(^{2+}\)-ATPase is to sustain elevated amounts of intracellular Mg\(^{2+}\) level in the brain; changes in the Mg\(^{2+}\) level in the brain can control the rate of protein synthesis and cell growth\(^3\). We witnessed reduced activity of Mg\(^{2+}\)-ATPase in group-II rats \((P < 0.001)\) when compared to those of group-I and group-III rats (Figure 8c). Group-IV rats showed no significant changes in the Mg\(^{2+}\)-ATPase activity when compared with those of group-I rats. Thus, it is clear that stress affects cell growth in the brain, while NJ could alter the effect.

5'-Nucleotidase is an ectoenzyme in myelin membranes and an intrinsic constituent of myelin. 5'-Nucleotidase is expressed in low levels in myelin membrane. 5'-Nucleotidase is considered to be involved in the transport of adenosine across the myelin membrane\(^4\). In the present study, the 5'-nucleotidase activity on myelin membrane showed reduction in group-II rats (Figure 8d). In group-I and group-III rats, there was significant increase \((P < 0.001)\) in the activity of 5'-nucleotidase whereas group-IV rats showed no significant changes in the 5'-nucleotidase activity on comparison with those of group-I rats.
Thus the effect on the ATPases highlights the efficiency of NJ in preserving the neural ionic homeostasis.

Acetylcholinesterase (AChE) is localized mainly at the neuromuscular junctions (NMJs) and cholinergic brain synapses whose function is to cease the synaptic (cholinergic) transmission. It rapidly hydrolyses acetylcholine (ACh) to liberate choline and acetate, therefore playing an important role in cholinergic pathways. Cholinesterase activity is limited to axons; however, it is also observed in the axonal vesicles of all unmyelinated and some of the myelinated nerve fibers. Ellman et al.\textsuperscript{16} reported that myelinated nerve fibers possess low levels of cholinesterase activity compared to unmyelinated nerve fibres. There are few reports available regarding the alterations of cholinergic system following stress. Anisman and McIntyre\textsuperscript{35} demonstrated reduced levels of Ach after stress. It has also been reported that AChE has a role in learning and memory processes; therefore, its disturbances in the basal FB cholinergic projections of hippocampus and other limbic structures were shown to impair learning and memory.\textsuperscript{16} This suggests that reduced cholinergic transmission might be partly accountable for cognitive deficits in IS group of rats. In the present study, AChE activity was found to decrease in group-II when compared with group-I, group-III and group-IV rats (Figure 9 a). Thus, NJ might be helpful in combating against IS-induced effects in AChE and their cognitive deficits. Interestingly, there was a significant increase in the activity of AChE in the FB region in all the groups than in the MB region of the rats.

Figure 8. Activities of ATPases (a–c) and 5′-nucleotidase (d) of control and experimental groups of rats. The activities are expressed as µmol/min/mg of protein and values are represented as mean ± SD.

Figure 9. Activity of acetyl cholinesterase (a) and levels of neurotransmitters, viz. dopamine (b) and serotonin (c) of control and experimental groups of rats. The activity and levels are expressed as µmol/min/mg of protein and ng/g of tissue respectively.
Dopamine is a neurotransmitter for neurons with cell bodies in MB. It is responsible for the feelings of pleasure and satisfaction; it also controls muscle function. Increased levels in the body lead to improper functioning of intestine, developmental delay and attention issues. A decrease in dopamine content leads to addictions and cravings. Stress induction elicits changes in dopamine content in the ventral tegmental regions, suggesting an uneven response of dopamine under different stressful stimuli. Chronic and uncontrollable or unavoidable exposure to immobilized stress could attenuate dopamine release. From Figure 9 b it can be inferred that there is a reduced level \((P < 0.05)\) of dopamine in the stress group of rats, but significant \((P < 0.01)\) increase in its level was observed in NJ-treated rats. However, group-IV rats show no significant changes in the level of dopamine when compared to those of group-I rats. Therefore, the results indicate that NJ has a neuromodulatory effect against stress, without affecting the normal pattern of neurotransmission in the brain.

Serotonin is a neurotransmitter which is produced in the raphe nucleus. It is considered to play a critical role in mood regulation. Serotonin is derived from L-tryptophan and controls the regulation of mood, behaviour, appetite and cerebral circulation. Texel et al. showed that induced stress affects the proper functioning of dopaminergic and serotonergic neurons. Interactions between serotonin and corticotrophin releasing factor (CRF) have been established by various studies on diverse regions of the brain. These studies have shown that reduction in serotonin level increases the sensitivity to stress. The present study showed impaired production of serotonin in stressed group of rats \((P < 0.05)\), whereas NJ group showed an increase \((P < 0.01)\) in serotonin content which is comparable to those of control (Figure 9 c). However, group-IV rats showed similar levels of serotonin as those of control. These observations show that NJ could play a role in stress responsiveness.

Cholesterol is a major lipid in the myelin membrane that helps maintain the membrane integrity. Figure 10 a shows the total content of cholesterol, which is found to be reduced \((P < 0.001)\) in stress-induced group when compared to those of group-I and group-III rats. However, group-I and group-IV rats showed no significant changes in myelin cholesterol level. NJ rats had showed a significant \((P < 0.001)\) increase in total cholesterol, indicating the existence of membrane integrity. Similarly, TLC staining of total lipid (Figure 11), cholesterol (Figure 12) and phospholipids (Figure 13) showed faint bands in IS group of rats. Groups I, III and IV exhibited dominant banding pattern.
The levels of phospholipid of the experimental group showed a significant ($P < 0.001$) reduction in IS group of rats and a substantially comparative ($P < 0.001$) increase in the level of phospholipid was found in control rats (Figure 10 b). Group-IV rats showed no significant changes in phospholipid content of their myelin.

Detection of ceramide (Figure 14) and glycolipid (Figure 15) also indicated loss of these compound lipids in stress-induced condition; however NJ could maintain the levels to a greater extent similar to those of normal animals. The FB region exhibited faint bands and the MB region showed high intensity of bands in all the groups, suggesting the presence of more compound lipids in the MB region of the animals.

In summary, IS exposure leads to increased oxidative stress in the FB and MB regions, as evident from increased lipid peroxidation in myelin membrane, which challenges the structural integrity and fluidity. As a result, there may be impaired conduction of the membrane-bound enzymes such as Na, K-ATPase, Ca-ATPase, Mg-ATPase and 5'-nucleotidase. This will have adverse effects on neural transmission, leading to the reduction in dopamine and serotonin levels. When the neurotransmitters are hampered, it results in psychological imbalance. By administering NJ to the stress-induced rats, its behaviour and the neurochemical status are found to be similar to those of the control animals, with no detrimental impact on the latter. The IS-induced rats were not found to be habituated to the imparted stress as evidenced by our results. Habituation, a complex phenomenon, is the resultant of various factors such as negative feedback mechanisms, duration and repetition of stress episodes, dishabituation, etc.41 The absence of habituation in the study might be due to the insufficiency of stress exposure required to pass the threshold beyond which habituation would occur.

This study showed that NJ (5 ml/kg body wt) could cause significant improvement in the behaviour of the stressed animals, and maintain normal levels of neurotransmitters and activities of membrane-bound ATPases as well as lipid profile of myelin in FB and MB. Earlier studies have reported NJ to be antioxidant42, anti-inflammatory43 and improve the endurance of athletes44. Our findings of biochemical, behavioural and neurotransmitter assessments demonstrated that NJ could significantly reduce the stress-induced variations in rats and thus mitigate health concerns associated with stress. We suggest that the juice of noni be subjected to validation by further investigation for elucidating its mechanistic effect against stress-induced condition.

**Conflict of interest.** The authors declare that there exists no conflict of interest. This work did not receive any financial assistance from any of the funding bodies.


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