CHAPTER 5

*MORINDA CITRIFOLIA* FRUIT JUICE FACILITATE THE MODULATION OF NEURAL-IMMUNE INTERACTIONS IN THE SPLEEN OF F344 MALE RATS

Specific Objective 3: To examine the effects of *Morinda citrifolia* fruit juice on neural-immune interactions in the spleen of aged F344 rats.

5.1 Rationale

Altered homeostatic functioning of neuroendocrine-immune network leads to the degeneration of noradrenergic (NA) nerve fibers in the secondary lymphoid organs and alter the neural-immune interactions. Age-related alterations in the cellular and molecular pathways also occur that leads to dysregulation of several cellular responses for the given stimuli in the neuronal and immune cells. Examining the altered neural-immune interactions in the spleen will help us to know the role of Noni fruit juice in regulating these interactions and molecular pathways involved in the aged population. Hence, the aim of this study is to investigate the effect of Noni (*Morinda citrifolia*) fruit juice (NFJ) on modulation of neural-immune interaction, cytokine production, compensatory mechanism and signaling molecules in spleen isolated from young and old F344 rats.

5.2 Treatment

5.2.1 Experiment

Young and old male F344 rats were obtained from the National Institute of Nutrition at Hyderabad for the study. The animals were acclimatized to the animal house at SRM University for a period of one week. After the period of acclimatization or/and after the completion of treatment period, animals were sacrificed by decapitation at 08:00 hrs and spleens were dissected and kept in sterile tubes containing HBSS. All animal experiments were conducted in accordance with the principles and procedures outlined and approved by the University’s Institutional Animal Ethics Committee.
5.2.1.1 Experiment 1

Young (3-4 months old; n=5) and old (18 months; n=7) aged male F344 rats were acclimatized to the animal house at SRM University for a period of one week. After the acclimatization period, animals were sacrificed by decapitation at 08:00 hrs and spleens were dissected and kept in sterile tubes containing HBSS for in vitro experiment with Noni (Morinda citrifolia) fruit juice (NFJ).

Noni (Morinda citrifolia) fruit juice (NFJ) was obtained from World Noni Research Foundation, Chennai and serially diluted in substituted RPMI medium from 1% to 0.01% and finally, to 0.0001 % and a dose response curve was generated. Splenic lymphocytes (2 x 10^5 cells/ml) were treated with different dilutions of Noni (Morinda citrifolia) fruit juice (NFJ) in 24 and 96 well-plates in the presence of 0 to 1.25 μg/ml of Con A and incubated in a humidified chamber with 5% CO₂ at 37°C to measure its effects on T lymphocyte proliferation, cytokines production, and expression of molecular markers.

5.2.1.2 Experiment 2

Young (3 months old; n=8) and old (16-17 months old, n=45) aged male F344 rats were obtained from the National Institute of Nutrition at Hyderabad for the study. The animals were acclimatized to the animal house at SRM University for a period of one week. After the period of acclimatization the old aged (16-17 months old) male F344 rats were randomly distributed into a control group (Old + Saline; n=13), and three Noni treated groups (Old+ 5% NFJ, n=11; Old+10% NFJ, n=11; and Old+20% NFJ, n=10). A separate group of 3 months old male rats (Young; n=8) served as control animals.

Noni (Morinda citrifolia) fruit juice (NFJ) was obtained from World Noni Research Foundation, Chennai. After the acclimatization period, Oral administration of Noni (Morinda citrifolia) fruit juice (NFJ) (5%, 10% and 20%) was done twice a day for a period of 60 days (5ml/kg body weight) in old F344 male rats. After the completion of treatment period, splenic lymphocytes (2 x 10^5 cells/ml) were isolated and incubated in the presence of 0 to 1.25 μg/ml of Con A in 24 and 96 well-plates and incubated in a humidified chamber with 5% CO₂ at 37°C to measure its effects on T lymphocyte proliferation, cytokine production, and expression of molecular markers.
5.3 Results

5.3.1 Effect of oral administration of Noni (*Morinda citrifolia*) fruit juice on body weight of F344 rats

A significant (p<0.05) age-associated decline in body weight of saline-treated old male F344 rats was observed in comparison to young rats. Although, treatment with NFJ did not have any significant effect in the body weight of old rats compared to saline-treated group (Fig. 5.1).

![Figure 5.1](image)

**Figure 5.1** Body weight of old F344 rats after 60 days of oral administration of NFJ. Age-associated decrease in the body weight of old rats were not reversed by treatment with NFJ. #<0.05 compared to Young.

5.3.2 Treatment with Noni (*Morinda citrifolia*) fruit juice enhanced Con A-induced splenocytes proliferation

A significant (p<0.05) age-associated decline in con A-induced proliferation of splenocytes (0.5 and 1.25 µg/ml Con A) was observed in old rats compared to young rats (Fig. 5.2A and 5.2B). *In vitro* incubation of splenocytes with 0.1% NFJ significantly (p<0.05) increased Con A-induced proliferative capacity of T lymphocytes in young rats (Fig. 5.2A). Similar to *in vitro study*, an age-related decline in con A-induced proliferation was observed in splenocytes isolated from old saline-treated rats compared with young rats (p<0.05). Splenocytes isolated from old F344 rats after 60 days of oral administration
of NFJ (10% and 20%) significantly (p<0.05) enhanced con A-induced lymphocyte proliferation compared to saline-treated old rats (Fig. 5.2B).

**Figure 5.2** Con A-induced proliferation of splenocytes by *in vitro* (A) and *in vivo* (B) treatment of NFJ. Con A-induced proliferation of splenocytes by *in vitro* treated splenocytes (2 x 10³ cells/well) with 0.1% dose of NFJ and splenocytes isolated from old rats after 60 days of oral administration of NFJ were incubated with 0, 0.5, 1.25, or 5 µg/ml of con A for 72 h and proliferation was measured by MTT assay. #p<0.05 compared to Young, p<0.05 Compared to age-matched control.
5.3.3 Treatment with Noni (*Morinda citrifolia*) fruit juice increased cell-mediated immune function

Age-related decline in IL-2 and IFN-γ production by the splenocytes was observed both *in vitro* and *in vivo* (Figs. 5.3A-D). *In vitro* incubation with NFJ significantly (p<0.05) increased IL-2 production by splenic lymphocytes from young (0.1%) and old (0.1%) rats (Fig. 5.3A). Similarly, long-term oral administration of NFJ to old rats in all doses (5%, 10% and 20%) reversed the age-related decline in IL-2 production by splenocytes (Fig. 5.3B). *In vitro* co-culturing of splenocytes with NFJ did not alter IFN-γ production in young rats while all the concentrations of NFJ (0.0001% to 1%) enhanced IFN-γ production in old rats (Fig. 5.3C). *In vivo* treatment of old rats with NFJ (5%, 10% and 20%) also resulted in preventing age-associated decline in IFN-γ production and increased its production by splenocytes (Fig. 5.3D).

*In vitro* incubation of splenocytes with NFJ (0.0001%, 0.001%, 0.01%, and 1%) significantly (p<0.05) increased the TNF-α production in splenocytes isolated from young and old F344 rats while *in vivo* treatment with NFJ did not have any significant effect in TNF-α production (Fig. 5.3E and 5.3F). Similar to TNF-α production, co-incubation with NFJ increased IL-6 production by splenocytes from young (0.1% and 1%) and old (1%) rats (Fig. 5.3G). In contrast, treatment of old rats with all 3 doses of NFJ for 60 days decreased IL-6 production compared to old saline-treated rats (Fig. 5.3H).
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Figure 5.3  Con A-induced production of cytokine [IL-2, IFN-γ, IL-6, and TNF-α] by splenocytes after in vitro (A, C, E and G) and in vivo (B, D, F and H) treatment with NFJ in young and old male F344 rats. Splenocytes were co-cultured with 1.25 μg/ml of Con A and NSJ for 24 h (A, C, E and G), Similarly splenic lymphocytes (2 × 10^5 cells/well) isolated from old F344 rats after the 60 days of oral administration of NFJ along with young and old control were co-cultured with 1.25 μg/ml of Con A (Figs. B, D, F and H) for 24 hrs and the supernatants were used for cytokine assays using ELISA kits.

# p<0.05 Compared to young, * p<0.05 Compared to age-matched control.

5.3.4. Effects of Noni (*Morinda citrifolia*) fruit juice on the expression of p-TH, NGF, p-mTOR, p-IκB-α and p-NF-κB (p50 and p65)

Age-related decline in expression of NGF was observed in saline-treated old rats compared to young controls (Figs. 5.4A and 5.4B). There was no significant (p<0.05) decline in the expression of p-mTOR, p-IκB-α and p-NF-κB (p50 and p65) in old rats compared to young animals.

Treatment with NFJ significantly (p<0.05) increases the expression of TH (10%) and NGF (10% and 20%) in old rats compared to saline treated rats. Treatment with NFJ did not have any significant effect in the expression of p-mTOR in old rats.

There was a significant (p<0.05) age-related increase in expression of p-IκB-α in the spleens of old rats compared to young rats (Figs. 5.4A and 5.4B). However, treatment with NFJ (5% and 10%) significantly (p<0.05) decreased the expression of p-IκB-α in old rats. Similarly, treatment with all 3 doses of NFJ (5%, 10% and 20%) significantly (p<0.05) decreased the expression of p-NF-κB (p50) in old rats compared to saline-treated
old rats while no significant difference were observed in the expression of p-NF-κB (p65) in old rats.

![Western immunoblots (A) probed with antibodies against p-TH, NGF, p-mTOR, p-IκB-α, p-NF-κB (p50) and p-NF-κB (p65) expression in the spleen isolated from old rats after 60 days of oral administration of NFJ. β-actin was used as internal control. Equal amounts of total protein (30 μg) were immunoblotted for the indicated proteins. Lower panel are the bar graphs (B) representing the relative density of the indicated proteins that were normalized with β-actin. *p<0.05 compared to Young, #p<0.05 compared to old saline.](image)

**Figure 5.4** Western immunoblots (A) probed with antibodies against p-TH, NGF, p-mTOR, p-IκB-α, p-NF-κB (p50) and p-NF-κB (p65) expression in the spleen isolated from old rats after 60 days of oral administration of NFJ. β-actin was used as internal control. Equal amounts of total protein (30 μg) were immunoblotted for the indicated proteins. Lower panel are the bar graphs (B) representing the relative density of the indicated proteins that were normalized with β-actin. *p<0.05 compared to Young, #p<0.05 compared to old saline.
5.3.5 Effects of Noni (*Morinda citrifolia*) fruit juice on age-related expression of p-ERK/Total ERK, p-CREB/Total CREB, and p-Akt/Total Akt in splenocytes

An age-associated decline in the expression of p-CREB/Total CREB and p-Akt/Total Akt in the splenic lymphocytes was observed both *in vitro* and *in vivo* (Figs. 5.5C-F) although the expression of p-ERK/Total ERK decreased only in old saline-treated rats (Fig. 5.5B).

*In vitro* incubation of splenic lymphocytes with NFJ significantly (p<0.05) enhanced the expression of p-ERK1/2/Total ERK and p-CREB/Total CREB in young (0.01% to 1%) and old (0.0001% to 1%) F344 rats (Figs. 5.5A and 5.5C). Similarly treatment with NFJ significantly (p<0.05) increased the p-Akt/Total Akt expression in the splenocytes isolated from young (0.0001% and 1%) and old (0.0001%, 0.01% and 1%) rats (Fig. 5.5E).

*In vivo* treatment with NFJ (10%) increased p-ERK1/2/Total ERK expression in old rats (Fig. 5.5B). Similarly, treatment with 5% dose of NFJ significantly (p<0.05) increased p-CREB/Total CREB expression (Fig. 5.5D) while 10% and 20% dose of NFJ significantly (p<0.05) increased the expression of p-Akt/Total Akt in old rats compared to saline-treated old rats (Fig. 5.5F).
B

- Young
- Old+Saline
- Old+5% NFJ
- Old+10% NFJ
- Old+20% NFJ

C

- Control
- 0.0001 % NFJ
- 0.001 % NFJ
- 0.01 % NFJ
- 0.1 % NFJ
- 1 % NFJ

D

- Young
- Old+Saline
- Old+5% NFJ
- Old+10% NFJ
- Old+20% NFJ
Figure 5.5  Expression of p-ERK/Total ERK, p-CREB/Total CREB and p-Akt/Total Akt in splenocytes after *in vitro* (A, C and E) and *in vivo* (B, D and F) treatment with NFJ in young and old F344 rats. #p<0.05 compared to young, * p<0.05 compared to age-matched control.
5.3.6 Treatment with Noni (*Morinda citrifolia*) fruit juice decreased the extent of lipid peroxidation

Age-associated increase in the extent of lipid peroxidation in the splenic lymphocytes was observed both *in vitro* and *in vivo* in old F344 rats (Figs. 5.6A and 5.6B). *In vitro* incubation of splenic lymphocytes with NFJ significantly (p<0.05) decreased the extent of lipid peroxidation in young (0.001%, 0.01% and 0.1%) and old (0.0001% to 1%) F344 rats (Fig. 5.6A). Similarly, *in vivo* treatment with NFJ (10% and 20%) significantly (p<0.05) decreased extent of lipid peroxidation in old rats (Fig. 5.6B).

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**Figure 5.6** The extent of lipid peroxidation in splenocytes after *in vitro* (A) and *in vivo* (B) treatment with NFJ in young and old F344 rats. *#*p<0.05 Compared to young, 
*# p<0.05 compared to age-matched control.*
5.3.7 *In vivo* treatment with Noni (*Morinda citrifolia*) fruit juice down-regulated the protein carbonyl formation

A significant (p<0.05) age-associated increase in protein carbonyl formation was observed in spleens of old rats (Fig. 5.7). However, treatment with NFJ (20%) significantly (p<0.05) decreased the protein carbonyl formation in spleen of old rats compared with old saline-treated rats.

![Graph showing protein carbonyl formation in spleen after oral administration of NFJ in old F344 rats](image_url)

Figure 5.7  Protein carbonyl formation in spleen after 60 days of oral administration of NFJ in old F344 rats. An age-related increase in protein carbonyl formation was reversed by *Morinda citrifolia* fruit juice (20%) in old rats compared to saline-treated rats.

# Compared to young, *Compared to age-matched control.
5.3.8 *In vivo* treatment with Noni (*Morinda citrifolia*) fruit juice increased nitric oxide (NO) production

Although no age-related decline in NO production was observed in culture supernatant isolated from splenocytes. Treatment with Morinda citrifolia fruit juice (10% and 20%) significantly (p<0.05) increased the NO production in splenocytes isolated from old rats compared with old saline-treated rats (Fig. 5.8).

**Figure 5.8** Nitric oxide (NO) production as measured using the Greiss reagent system in in spleen after 60 days of oral administration of NFJ in old F344 rats. *Compared to age-matched control.*
Figure 5.9  An overview of the results depicting the probable intracellular pathways involved in mediating immunomodulatory and anti-inflammatory effects of Noni (Morinda citrifolia) fruit juice (NFJ) in splenic lymphocytes of F344 rats. Abbreviations; TH-Tyrosine hydroxylase, NGF- Nerve growth factor, ERK- Extracellular signal regulated kinase, Akt- Protein kinase B, CREB- cAMP response element binding protein, CBP-CREB binding protein, CRE- cAMP response element, IκB- α-Inhibitor of kappa B, IKK-IκB kinase, NF-κB- Nuclear factor kappa-chain-enhancer of activated B cells, ROS- Reactive oxygen species
5.4 Discussion

In this study, we have examined the effects of Noni (*Morinda citrifolia*) fruit juice (NFJ) on age-related alterations in immune function, expression of molecular markers, neuronal factors involved in neural-immune interactions, and antioxidant enzyme activities in the spleen from young and old F344 rats. Results from our study show that NFJ modulates the immune functions in old rats, in a manner similar to young rats possibly through upregulation of cell-mediated immunity in the splenocytes. These alterations in the immune system were accompanied by increasing the expression of TH and NGF, upregulation the expression of cell signaling markers, ERK, CREB, and Akt, and decreasing the oxidative stress in splenocytes isolated from F344 rats.

Age-dependent decrease in lymphocyte proliferation in response to mitogen such as concanavalin A has been examined by a number of animal and clinical studies [32, 50, 67]. Treatment with NFJ modulates immune function by increasing the lymphocytes proliferation and cytokine (IL-2 and IFN-γ) production in young and old F344 rats. Likewise, hydroalcoholic and aqueous extracts of *Morinda citrifolia* increased splenocytes proliferation and delayed-type hypersensitivity reaction and thus, emphasizing its immunostimulatory properties [128]. Age-related decline in the proliferation of lymphocytes also correlates with decrease in IL-2 production [53].

*In vitro* treatment with fruit juice increased the IL-6 and TNF-α production, although chronic *in vivo* treatment with fruit juice decreased the IL-6 production in old rats. *Morinda citrifolia* fruit extract modulate the cell-mediated immune function by inhibiting the decrease in IL-2 production in BALB/c mice [136]. Studies from our and other laboratories have illustrated the immunomodulatory effect of Noni fruit juice in the lymphocytes isolated from young and old rats [41, 128, 132].

Dendritic cells treated with fermented Noni exudate stimulates the proliferation of splenocytes isolated from C57BL/6J mice [129]. Our finding is consistent with the hypothesis that aging causes a decline in cytokine production (IL-2 and IFN-γ) from Th1 lymphocytes and an increase in cytokine production (IL-4 and IL-6) from Th2 lymphocytes [27]. Results from our study show that NFJ has the capacity to modulate the immune system in old rats, in a manner that is similar to young rats possibly through regulation of cell-mediated immunity in the splenocytes.
Damnacanthal isolated from the root of *Morinda elliptica* shows the immunomodulatory effects by increasing the lymphocytes proliferation and cytokine production in mouse thymocytes and human peripheral blood mononuclear cells (PBMCs) [137]. Noni fruit juice promotes an increase in cell mediated immune response through the activation of CB2 receptors that is capable of not only enhancing IFN-\(\gamma\) production, but also through the suppression of Th2 cytokine (IL-4) [123]. Furthermore, age-associated decline in immune dysfunction also correlates with an increase in Foxp3+ Treg cell population in spleen of old mice leading to decline in co-stimulation signals from the dendritic cell [138]. NFJ mediated concomitant increase in IL-2 and IFN-\(\gamma\) production can also be due to the presence of lectin in fruit juice, as a study shows that lectin isolated from garlic stimulates the production of IFN-\(\gamma\) in splenocytes and upregulates the IL-12 production via activation of p38-MAPK and ERK pathways [139].

In old rats, Noni (*Morinda citrifolia*) fruit juice partially restores the age-related decline in expression of TH and NGF in the spleen suggesting its profound role as an immunomodulator and a neurotrophic factor. Age-associated decline in TH expression might be due to the loss of NA innervations in the spleen of old animals that may have occurred from the accumulation of toxic oxidative metabolites of NE and free radicals in the local microenvironment. Chemical [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4)] induced alteration in cytokine production in the spleen is due to degeneration of noradrenergic neurons in the locus coeruleus (LC) and the A5 cell group mediated by decrease in TH (+) nerve fibers [140].

The age-associated loss of sympathetic NA innervation in the secondary lymphoid organs (spleen and lymph nodes) follow a similar pattern and therefore, similar factors such as growth factors, hormones, and other compensatory mechanism may be responsible for the degeneration observed in these organs with advancing age [25]. The role of NFJ in upregulation of age-dependent decrease in TH and NGF expression and cytokine production in the absence of any other external source might be due to revival of the growth of NA fibers in the spleen and by up-regulating various intracellular signaling pathways. Several studies have examined the role of NGF in modulation of immune system in animals and humans [35, 141]. A study conducted on peripheral blood mononuclear cells (PBMCs) revealed that stimulation of lymphocytes isolated from PBMCs with NGF increased the expression of the IL-2 receptor [142].
Nerve growth factor (NGF), an essential neurotrophic factor for the survival of sympathetic neurons, is extensively distributed in the parenchyma of the spleen and can stimulate the proliferation of T and B cells, enhance antibody production, and facilitate lymphocyte migration [143]. Several other medicinal plant extracts and phytochemicals have been found to have neuroprotective effects by upregulating the expression of nerve growth factor [132, 144, 145].

NFJ treatment in old rats, downregulated the expression of p-NF-κB (p50) by inhibiting the phosphorylation of I-κB-α. Morinda citrifolia fruit juice-mediated decline in p-NF-κB expression observed in our study can be due to the presence of damnacanthal and other phytochemicals that possess anti-inflammatory property and also regulate the expression of proinflammatory cytokines and inducible nitric oxide synthase [41, 131, 146]. Results from these findings provide a potential mechanism through which Morinda citrifolia mediates its anti-inflammatory effects.

Similar to damnacanthal, quercetin in Noni fruit extract exhibits its anti-inflammatory property through the suppression of the intracellular reactive oxygen species, COX-2, IL-8, and prostaglandin E2 production by preventing the translocation of NF-κB p65 to the nucleus in colon cancer cell line [147]. Methanolic extract of the roots of Morinda officinalis inhibits NF-κB expression and TNF-α production in LPS-stimulated macrophages [132]. As for the supportive evidence, treatment with monotropin isolated from Morinda officinalis inhibits LPS-induced activation of NF-κB via degradation of IκB-α by suppressing the IKK kinase activity in RAW 264.7 macrophages cell line. Monotropein also inhibited the dextran sodium sulfate (DSS)-induced IκB-α phosphorylation and the subsequent nuclear translocations of NF-κB (p50) subunits in inflamed colonic tissues while also inhibiting signaling cascades of inflammation [148].

NFJ decreased the extent of lipid peroxidation and protein carbonyl formation in spleen isolated from old F344 rats. A decrease in the extent of lipid peroxidation and protein carbonyl formation indicates inhibition in the generation of reactive oxygen species and free radicals that might be generated from the metabolism of biogenic amines. The role of NFJ in upregulating the NGF expression and immune responses suggests that it is capable of restoring splenic NA fibers and thus, neural-immune interactions may depend on these factors and also, enhancement of antioxidant activities and through various intracellular signaling pathways.
In vitro and in vivo treatment with NFJ increased the expression of p-ERK and p-CREB in a dose-dependent manner in young and old rats. Age-associated decline in activation of MAP kinase proteins in stimulated T lymphocytes has been observed in a number of studies [149, 150]. NFJ-mediated increase in expression of molecular markers can be due to some of the metabolites generated from the digestion of Noni fruit juice. Age-dependent decrease in ERK expression has been observed in spleen isolated from old F344 rats (41, 151).

Age-associated alteration in MAPK pathways might have been responsible for the decrease in IL-2 production and lymphocytes proliferation. Clinical and animal studies have shown that defects in ERK 2 pathways can lead to a decrease in lymphocyte proliferation and IL-2 production [151, 152]. Remarkably, NFJ-mediated increase in IL-2 and IFN-γ production was accompanied by the activation of p-ERK/p-CREB pathways leading to increase in cell-mediated immune function.

Studies from our laboratory have shown that plant extract like Brahmi can reverse the age-associated decline in immune function by upregulation of cytokine production and intracellular signaling pathways such as ERK and CREB in splenocytes isolated from rats [56, 57]. As evident from our results, that Noni fruit juice-mediated increase in expression of nerve growth factor might have been mediated through ERK/CREB pathways. Similar to ERK and CREB, Noni fruit juice-mediated increase in Akt expression was observed in young and old F344 rats. Although there was a tendency of mTOR expression to decline in the spleens of in NFJ-treated old rats, it was not statistically significant.

There was a tendency for NO production to decline with age in splenocytes similar to alveolar macrophages that may alter vasodilation resulting in impaired trafficking of immune cells [153]. In contrast, treatment of old rats with NFJ enhanced NO production that may exert immunostimulatory properties through cGMP-mediated Akt activation [154]. The expression of mTOR in the spleen showed a trend to decrease in old rats following in vivo treatment with NFJ that may explain improved intracellular signaling in splenocytes and possibly aid in reversing age-related deficits in immunity [48, 49].

In summary, we conclude that Noni (Morinda citrifolia) fruit juice has beneficial effects on immune function by increasing the cytokine production, intracellular signaling markers and decreasing the expression of inflammatory markers. Treatment with Noni (Morinda citrifolia) fruit juice enhanced the neural-immune interaction by upregulating the expression of TH and NGF that might have been due to increase in NA innervations in the spleen of old F344 rats.
5.5 Key findings

Treatment with NFJ modulated cell-mediated immune function by upregulating lymphocyte proliferation and Th1 cell-mediated IL-2 and IFN-γ production in splenocytes of young and old F344 rats.

In old rats, NFJ partially reversed the age-related decline in TH and NGF expression in the spleen, suggesting that it has a profound role as a neurotrophic factor and thus, facilitating the expression of tyrosine hydroxylase. The increase in the expression of TH suggests possible reinnervation of sympathetic neurons in the spleen. NFJ downregulated the expression of inflammatory markers p-mTOR and p-NF-κB (p50) by inhibiting the phosphorylation of I-κB-α in old rats.

In spleen, expression of molecular markers was differentially regulated by different doses of Noni fruit juice. *In vitro* and *in vivo* treatment with NFJ increased the expression of p-ERK, p-Akt and p-CREB in a dose dependent manner in young and old rats.

In summary, results from *in vitro* and *in vivo* effects of *Morinda citrifolia* fruit juice (NFJ) suggest that treatment with NFJ reverses the age-associated decline in neural-immune interactions by increasing the cell-mediated immune function, upregulating the expression of neural factor, and decreasing the expression of inflammatory markers [p-IκB-α; p-NF-κB (p50)] in old F344 rats.