ABSTRACT

Background

Population aging is a worldwide phenomenon, and globally, the number of old people is expected to increase in both developed and developing countries. Aging is a natural process accompanied by a loss of neuroendocrine-immune homeostasis and the ensuing deterioration of physiological functions that lead to the development of several age-associated diseases such as cancer, Alzheimer’s disease, and Parkinson’s diseases. As the rate of aging increases, the prevalence and clinical importance of infirmity is expected to increase worldwide. Therefore, understanding the basic cellular and molecular mechanisms involved in the aging process is crucial to develop effective strategies in preventing the development of age-related diseases.

Several studies from our and other laboratories have reported a functional basis for the link between the three homeostatic systems, namely nervous, endocrine, and immune systems and their role in health and diseases. With advancing age, there is an increase in the level of reactive oxygen species (ROS) and decrease in noradrenergic (NA) innervation in lymphoid organs that may be responsible for suppressed immunocompetence during aging. Parallel to age-associated decline in immune functions, alterations occurs in the brain, increasing susceptibility of infections and age-related diseases, such as neurodegenerative disorders.

We have understood that aging cannot be reverted but it can be slowed by maintaining a healthy lifestyle, and by the inclusion of intervention therapies that can remedy an unhealthy lifestyle. A number of intervention therapies such as exercise, yoga, meditation have been investigated extensively for the promotion of healthy aging. In traditional culture and Ayurveda, various plant extracts have been identified and experimented and several of them have been found to have beneficial health effects. Hence, assessing how extracts from plant like Morinda citrifolia can modulate the neural-immune network could play a vital role in providing natural remedies to prevent age-associated dysfunctions in the old population. Morinda citrifolia, also commonly referred to as Noni, has been used widely since ancient times in the traditional medicine because its constituents possess multiple biological activities and thus, exert curative effects on a number of diseases such
as inflammatory diseases, diabetes, cardiovascular diseases, infectious diseases, cancer, and cognitive disorders. Although there are several findings, discussing about the pharmacological functions and clinical benefits of the *Morinda citrifolia* at the cellular level in various diseases, not much emphasis has been placed on understanding the role of its phytoconstituents in healthy aging. This necessitates the need for further studies to explore the mechanisms by which *Morinda citrifolia* and its phytochemicals mediate its effects on physiological functions.

We hypothesize that *Morinda citrifolia* fruit juice modulates neuroendocrine-immune interactions and compensatory mechanisms in the aged population for the maintenance of homeostasis.

**Objectives**

The following objectives have been formulated to test our hypothesis.

1. To investigate the dose response of *Morinda citrifolia* fruit juice on lymphoproliferation and antioxidant enzyme activities in splenic lymphocytes of young rats.
2. To examine the effects of *Morinda citrifolia* fruit juice on age-associated modulation of immune functions and the possible intracellular molecular targets influenced by Noni using Bioinformatics tool.
3. To examine the effects of *Morinda citrifolia* fruit juice on neural-immune interaction in the spleen of aged F344 rats.
4. To investigate the role of *Morinda citrifolia* fruit juice on age-associated alteration of neuroendocrine functions, growth factors, and signaling mechanisms in various brain areas of old F344 rats.

**Methods**

Briefly, lymphocytes were isolated from the spleen of young male Wistar rats and treated with different types of Noni fruit juice [Noni (*Morinda citrifolia*) fruit juice (NFJ), Noni fruit juice with seeds (NWS) and Noni seedless fruit juice (NSL)] in various dilutions ranging from 0.01 to 10% (0.01%, 0.1%, 1%, 5%, 10%) for 24, 48, and 72 hrs in order to establish the dose response and incubation time of Noni fruit juice on splenocytes proliferation and antioxidant status.
In *in vitro* study, lymphocytes were isolated from the spleens of young, middle-aged, and old male F344 rats and treated with different types of Noni fruit juice (NFJ, NWS, and NSL) in RPMI medium from 1% to 0.01% and finally to 0.0001 % for the examination of immune function, intracellular signaling markers and compensatory mechanisms.

Using Bioinformatics tools (*Auto Dock4.2.6*) molecular docking was performed by keeping the ERK molecule rigid and ligand flexible (Rigid Docking). This resulted in different conformations in each run and the best conformer, which fit with lowest binding energy (kcal/mol) was chosen and written as a PDB file using UCSF Chimera. *Auto Dock4.2.6* was used to automatically dock the ligands to the enzyme. The docked structures of the inhibitors were generated after the maximum number of evaluations using UCSF Chimera.

In *in vivo* study, old F344 rats were treated by oral administration of Noni (*Morinda citrifolia*) fruit juice (NFJ) (5%, 10% and 20%) twice a day for a period of 60 days (5 ml/kg body weight). After the completion of treatment period, spleens and brain areas (frontal cortex, medial basal hypothalamus, striatum, and hippocampus) were dissected and stored for further assays.

**Results**

All three types of Noni fruit juice [Noni (*Morinda citrifolia*) fruit juice (NFJ), Noni fruit juice with seeds (NWS), and Noni seedless fruit juice (NSL)] significantly enhanced splenocyte proliferation, and differentially regulated activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and the extent of lipid peroxidation. Although the results varied among the doses and types of fruit juice studied for the lymphocytes proliferation and antioxidant enzyme activities, the increase in lymphocytes proliferation observed with different Noni fruit juice could also be due to the presence of lectin in the fruit juice.

Treatment with NWS was found to decrease SOD, CAT and GPx activities and reversed the extent of lipid peroxidation. NSL increased SOD and CAT activities, but it decreased in GPx activity and increased extent of lipid peroxidation. NWS enhanced lymphoproliferation in young and old rats, and IL-2 and IFN-γ production in middle-aged and old rats. NWS decreased the age-related increase in extent of lipid peroxidation in all age-groups; however treatment with NSL increased the extent of lipid peroxidation in old F344 rats. Thus, it can be concluded that Noni fruit juice has antioxidant and immune-
enhancing properties. Treatment with NSL increased the expression of p-ERK in old rats and downregulated the expression of p-CREB in young and middle-aged rats. In contrast, NWS treatment decreased the expression of p-ERK in young, middle-age and old rats and increased the expression of p-CREB in old rats alone. Both NSL and NWS increased p-Akt expression and decreased the expression of p-NF-κB in middle-aged and old group. Results from docking study showed that the phytochemicals present in Noni, such as damnacanthal, myricetin and ursolic acid, are the potent inhibitors of ERK with binding affinity in catalytic and phosphorylation sites of the molecule.

Noni (Morinda citrifolia) fruit juice (NFJ) significantly increased Con A-induced lymphocytes proliferation, IL-2 and IFN-γ production, and expressions of p-ERK, p-Akt, p-CREB in young and old rats, although the differential effects were observed on IL-6 and TNF-α production. In vivo treatment with NFJ increased the expression of tyrosine hydroxylase (p-TH), nerve growth factor (NGF), and nitric oxide (NO) production and suppressed the expression of I-κB-α and p-NF-κB (p50) in splenocytes of old rats.

Treatment with Noni (Morinda citrifolia) fruit juice (NFJ) increased serum testosterone levels and decreased serum corticosterone levels in old rats. Similarly, NFJ upregulated brain derived growth factor (BDNF) and insulin like growth factor-1 (IGF-1) levels and decreased amyloid precursor protein (APP) levels in various brain areas [frontal cortex (FC), striatum (STR), medial basal hypothalamus (MBH), hippocampus (HP)] of old rats. NFJ treatment increased the expression of molecular markers (p-ERK, p-CREB and p-Akt), antioxidant enzyme activities and nitric oxide production in various brain areas, while differentially regulating the cholinesterase activities.

Conclusions

Taken together, these results suggest that Morinda citrifolia fruit juice enhanced cell-mediated immune responses, improved neural-immune interaction, and cell survival pathways that may be beneficial as intervention therapy in the aged population for promoting healthy aging. NFJ may promote remodeling of neuronal circuitry by differentially modulating the intracellular markers and neural factors, facilitating the use of Noni fruit juice as an avenue for the treatment of age-associated neurodegenerative disorders in the aged population. Further studies are needed to explore the link between Morinda citrifolia fruit juice-induced changes in different subsets of immune cells and in cognitive functions.