CHAPTER 3

ESTABLISHMENT OF DOSE RESPONSE OF *MORINDA CITRIFOLIA* FRUIT JUICES (NFJ, NSL, NWS) ON LYMPHOPROLIFERATION AND ANTIOXIDANT ENZYMES IN SPLENIC LYMPHOCYTES OF YOUNG MALE WISTAR RATS

Specific Objective 1: To investigate dose response of *Morinda citrifolia* fruit juices on lymphoproliferation and antioxidant enzyme activities in splenic lymphocytes of young rats.

3.1 Rationale

Immunosenescence and subsequent increase in the incidence of diseases may be the result of oxidative stress due to increased cellular insults and damage over the years by the formation of free radicals. The aim of this objective is to establish the ideal dose of different types of Noni fruit juice on splenic lymphocytes from young male Wistar rats to examine its effects on the lymphoproliferation and antioxidant enzyme activities. For this purpose, splenic lymphocytes isolated from young male Wistar rats were incubated with various type of Noni fruit juice for different time period and examined to get the ideal doses and incubation time for further investigating the effects of Noni fruit juice on physiological functions. The present study provides crucial evidence for the dose at which noni fruit juice exerts immunostimulatory effects and on antioxidant enzyme activities in the lymphocytes.

3.2 Treatment

3.2.1 Experiment

Splenocytes isolated from young male Wistar rats were treated with different types of the Noni fruit juice [Noni (*Morinda citrifolia*) fruit juice (NFJ), Noni fruit juice with (NWS) and without seeds (NSL)] at various concentrations ranging from 0.01% to 10% in RPMI media supplemented with concanavalin A for a period of 24 hrs, 48 hrs, and 72 hrs.
3.3 Results

3.3.1. Effects of Noni (*Morinda citrifolia*) fruit juice (NFJ), Noni fruit juice with seed (NWS) and Noni seedless fruit juice (NSL) on Con A-induced lymphocyte proliferation

*In vitro* incubation of splenocytes from young rats with 0.01%, 0.1% and 10% doses of Noni (*Morinda citrifolia*) fruit juice (NFJ) significantly (*p*<0.05) increased the proliferation of lymphocytes after 48 and 72 hrs of treatment (Fig.3.1A).

*In vitro* incubation of splenocytes with Noni fruit juice with seed (NWS) significantly (*p*<0.05) increased the proliferation of lymphocytes isolated from spleen in all doses after 24 hrs. Proliferation further increased after 48 hrs in 0.01%, 0.1%, 1%, and 10% doses of NWS and by all doses of NWS after 72 hrs of treatment (Fig. 3.1B).

*In vitro* incubation of splenocytes with NSL (0.1% and 10%) significantly (*p*<0.05) increased the proliferation of splenocytes after 48 hrs and by all doses of NSL after 72 hrs of treatment (Fig.3.1C).
Figure 3.1 Con A-induced proliferation of splenic lymphocytes. Splenic lymphocytes (2 x 10^5 cells/well) were incubated with different doses (0.01%, 0.1%, 1%, 5% and 10%) of NFJ (A), NWS (B) and NSL (C) for 24, 48 and 72 h. Lymphocytes proliferation was measured using MTT assay. *p<0.05 compared to age-matched control.
3.3.2. Effects of Noni (*Morinda citrifolia*) fruit juice (NFJ), Noni fruit juice with seed (NWS) and Noni seedless fruit juice (NSL) on Con A-induced antioxidant enzyme activities and extent of lipid peroxidation

3.3.2.1 Superoxide Dismutase (SOD)

Treatment with Noni fruit juice (NFJ and NSL) (5% and 10%) significantly (p<0.05) increased superoxide dismutase (SOD) activity (units/min/mg protein) (Figs. 3.2A and 3.2C), although all dose of NWS significantly (p<0.05) decreased the SOD activity in splenocytes isolated from young male Wistar rats (Fig. 3.2B).
3.3.2.2 Catalase (CAT)

Treatment with NFJ (0.01%) and NSL (1% and 5%) significantly (p<0.05) increased catalase (CAT) activity (units/min/mg protein) in splenocytes isolated from young Wistar rats (Fig. 3.2D and 3.2F), although treatment with 10% dose of NFJ significantly (p<0.05) decreased the catalase activity. Likewise, treatment with NWS (0.1% and 10%) significantly (p<0.05) decreased in catalase activity in splenocytes isolated from young rats compared to young control (Fig. 3.2E).
3.3.2.3 Glutathione Peroxidase (GPx)

Treatment with NWS (0.01%) showed a significant (p<0.05) increase in glutathione peroxidase (GPx) activity (units/min/mg protein) in splenocytes isolated from young Wistar rats (Fig. 3.2H). However, co-incubation of splenocytes with NFJ (1%, 5% and 10%) and NWS (5% and 10%) significantly decreased GPx activity in young male Wistar rats (Figs. 3.2G and 3.2H). Treatment with NSL did not have any effect on GPx activity in young rats (Fig. 3.2 I).

Figure 3.2 Effects of NFJ, NWS, and NSL on cellular antioxidant status. Antioxidant status of splenic lymphocytes from young male Wistar rats treated with NFJ, NWS and NSL. *p<0.05 compared to age-matched control.
3.3.2.4 Extent of lipid peroxidation

Treatment with NFJ and NWS (Fig. 3.3A and 3.3B) did not show any significant effect on the extent of lipid peroxidation (MDA equivalents/mg protein) in splenocytes isolated from young male Wistar rats. Although treatment of splenic lymphocytes with NSL (10%) significantly (p<0.05) increased the extent of lipid peroxidation in young male Wistar rats (Fig. 3.3C).

![Figure 3.3](image-url)

**Figure 3.3** Effects of NFJ, NWS, and NSL on the extent of lipid peroxidation. Extent of lipid peroxidation of splenic lymphocytes from young male Wistar rats treated with NFJ, NWS and NSL. *p<0.05 compared to age-matched control.
Figure 3.4  An overview of the results depicting immunomodulatory and antioxidative effects of Noni fruit juices on splenic lymphocytes *in vitro*. 
3.4 Discussion

This study was conducted with the aim to establish the ideal dose of *Morinda citrifolia* fruit juices on splenic lymphocytes and to examine the lymphoproliferation and antioxidant enzyme activities in young male Wistar rat. The purpose of using three types of noni fruit juices (NFJ, NWS, and NSL) was to investigate the effects of different types of fruit juice on immune function and other compensatory mechanism on splenic lymphocytes. In this was a preliminary study, three types of fruit juices were selected to account for the differences in its effects due to the variability in the type of phytochemicals present in noni fruit and its seeds.

Results from the preliminary study conducted on young male Wistar rats showed that all three types of fruit juice [Noni (*Morinda citrifolia*) fruit juice (NFJ), Noni seedless fruit juice (NSL), and Noni fruit juice with seeds (NWS)] significantly enhanced splenocytes proliferation, and differentially regulates antioxidant enzyme activities and the extent of lipid peroxidation. Antioxidant enzymes play a crucial role in preventing the deleterious effects of free radicals from damaging the cellular structure and functions in the body. Plant extracts are known to be rich in flavonoids and other free-radical scavengers that contribute to their antioxidant properties [59]. Although the results varied among the doses and types of fruit juice studied for the lymphocyte proliferation and antioxidant enzyme activities.

The increase in lymphocyte proliferation observed with different Noni fruit juice could also be due to the presence of lectin in the fruit juice. Since Noni plant extracts are known to have several active molecules such as xeronines, terpenes, anthraquinones, scopoletin, morindone, morindin, asperuloside, acubin, caproic acid, caprylic acid, damna-canthal, polysaccharide, and alkaloids, it is possible that these phytochemicals may have contributed to its immunomodulatory effects [59, 123]. However, further studies are required to establish the role of individual components may be responsible for the proliferative effects observed in splenic lymphocytes.

Superoxide dismutase (SOD) and catalase (CAT) activity was significantly decreased in splenocytes treated with NWS, while treatment with NSL and NFJ significantly enhanced SOD and CAT activity compared to control. It is possible that certain phytochemicals present in the Noni fruit seeds are responsible for the inhibition the enzyme activity *in vitro* which warrants further examination.
Phytochemicals present in the Noni fruit juice may have contributed to the enhanced ROS scavenging ability by enhancing the activity of antioxidant enzymes, such as superoxide dismutase and catalase. Finally, glutathione peroxidase (GPx) activity was significantly decreased by treatment with NWS and NFJ; however treatment with NSL did not have any effect on the GPx activity. Thus, Noni fruit juice enhances the enzyme activities of SOD, CAT, and GPx and improves the antioxidant status by clearance of free radicals which may have been responsible for improved immune functions [124-126].

NSL alone significantly enhanced the extent of lipid peroxidation in splenocytes in the highest dose while treatment with NFJ and NWS did not significantly alter it when compared with young control. Although in our study we did not get a significant difference in the extent of lipid peroxidation except the 10% dose of NSL that may be due to the age of the rats which were young in this study.

Thus it can be concluded that Noni fruit juice may contribute to beneficial effects on immune functions by increasing lymphocyte proliferation and enhancing compensatory mechanisms such as the activities of antioxidant enzymes and/or through possible lectins that may also contribute to the same effect. Further studies are essential to examine the role of Noni fruit juice on the immune network and its implications in healthy aging.

### 3.5 Key findings

Results from the preliminary study conducted on young male Wistar rats showed that 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)] had profound effects on lymphocytes proliferation, antioxidant enzyme activities and the extent of lipid peroxidation.

The effects on lymphocytes proliferation and antioxidant enzyme activities differed among the doses and type of fruit juice which might be due to differences in the phytochemicals present in various fruit juices used in the study. Noni fruit juice may contribute to enhancing the immune function by increasing the antioxidant enzyme activities and reducing the age-dependent increase in free-radicals. Thus, it is important to examine the extent of influence exerted by Noni phytochemicals in modulating the bidirectional communication between the neuroendocrine system and immune system to alter the aging process.