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Review

The immunomodulating properties of noni juice

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Abstract: *Morinda citrifolia* (noni) fruit juice has the potential to influence immune system function. This review discusses results from several human and animal studies that provide insight into the potential mechanisms of action by which noni juice exerts its immunomodulatory effects. Increased natural killer cell activity is a likely a major contributor to the improved health outcomes and increased survival times described in case reports and as observed in LLC and S180 tumor bearing mice. Increased interferon-gamma (IFN-γ) production is also an important mechanism of action through which noni improves immune function. IFN-γ promotes natural killer cell activity and phagocytosis, activities both seen in human and bovine studies as well as in rodents. Noni promotes regulatory cytokine expression, such as IL-2 which stimulates CD4+ T cell differentiation. Noni juice appears to influence this process via kinase 1/2 (ERK1/2) protein kinase B (Akt) and nuclear factor-kappa-beta signaling. As oxidative status is known to influence immune function, this review also discusses the notable antioxidant properties of noni juice that have been demonstrated in human trials.

Keywords: Noni juice; *Morinda citrifolia*; immune system; immunomodulation

1. Introduction

Morinda citrifolia is a small to medium sized (3–10m high) fruit-bearing tree that grows throughout the tropics. The common names for this plant are Indian mulberry and noni, with the latter being more frequently used since the global market introduction of noni fruit juice products in the mid 1990's. Noni fruit and leaves have long been consumed as a traditional food by Pacific Islanders and in Southern and Southeast Asia [1]. The first academic description of the use of noni as a food was in 1769 by Sydney Parkinson, a naturalist sailing with Captain James Cook, who recorded that Tahitians ate noni fruit [2]. Rarotongans also ate the fruit often, and the Burmese used it to prepare curries [3,4]. The indigenous people of Australia ate noni fruit during the cool-dry season from May to August in the Northern Territory [5,6]. In Java and Thailand, noni leaves were eaten either raw or cooked [7]. Tahitian also wrapped fish in the leaves to impart an appealing flavor to the cooked fish [8].

The noni plant was reportedly the most important and widely used Polynesian medicinal plant prior to the arrival of Europeans with the fruits being used to treat diverse ailments, including infections [9]. More recently, the fruit is used to produce a juice that is often consumed to improve overall health. One major source of this juice is French Polynesia where noni fruit puree constitutes one of the largest agricultural exports [10]. In fact, more than 21,000 metric tons of this puree was exported in the past decade [11]. The global popularity of the juice is due to its perceived health value. Indeed, European consumers of a Tahitian-sourced noni juice beverage reported that increased energy, improved well-being, reduction of pain, fewer infections, improved sleep, improved digestion, reduction in allergy and asthma symptoms were the most frequently experienced health benefits [12].

Among the potential health benefits of noni juice is the modulation of immune system function. The Chinese government has approved one commercial source of noni juice, Tahitian Noni® Juice (TNJ), as a safe new resource and approved it as a functional food

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that can enhance immunity [13]. This review summarizes and discusses in vivo and human studies which demonstrate the ability of noni juice to directly influence immune system function. It is important to note that the antioxidant properties of noni juice provide additional evidence for immunomodulation activity, as there is well known connection between oxidative processes and the immune system. Reactive oxygen species (ROS) are involved in the modulation of immune processes, which include roles as secondary messengers during signal transduction between cells [14]. Multiple studies have demonstrated the ability of antioxidants, including fruits and fruit juices, to modulate immune responses [15-18]. Epidemiology studies have revealed an association between a lowered incidence of cancer and diets rich in antioxidant nutrients, for which antioxidant-induced improved immune function is suggested as an important factor [19]. Antioxidants have also improved immune function in the elderly [20]. As such, human studies demonstrating the antioxidant properties of noni juice are also discussed in this review.

2. Human Studies

The impact of noni juice on health has been observed and evaluated in human subjects. Studies relevant to immune system function are discussed below and summarized in Table 1.

Table 1. Human studies of noni juice and potential immunomodulation.

Study population	No. in study	Noni juice dose	Duration	Outcome	Reference
Cancer patients (case reports)	2	Not reported	6 months (case 1); unknown (case 2)	Extended survival time	Wong 2004 [21]
Health adults	12	330 mL (TNJ)	8 weeks	Increased IL-2 and NK cell activity	Ma et al. 2008 [22]
General population (survey)	408	Unspecified	Only during length of illness as well 2 to 10 years	Traditional use of noni juice to treat diseases associated with respiratory infections	Pande et al. 2005 [23]

2.1. Case Reports

The immunomodulating properties of noni juice were discussed in a report of two clinical case studies [21]. The investigating physician examined the cases of two cancer patients in which the use of noni juice was reported to have been instrumental in disease control over many years. Medical records, X-ray and biopsy slides were reviewed with a radiologist and pathologist. In-depth interviews with relatives were also conducted.

The first case was a 69-year-old male diagnosed with diffuse adenocarcinoma, after he experienced anorexia, epigastric discomfort and weight loss. He had refused surgery and left the hospital. During the subsequent two months, his weight dropped from 165 to 70 pounds (74.8 to 31.7 kg), and he was only able to retain liquid foods, with intermittent vomiting. At this time, the patient decided to take noni juice. His health began to improve remarkably. Within one month he was able to eat a regular diet and began to regain his weight. He was also able to resume hunting and fishing activities. For six months, the patient drank noni juice regularly and was discharged from hospice care one year after being admitted. At discharge, X-rays were normal. Approximately six years after the initial adenocarcinoma diagnosis, the patient underwent a follow-up endoscopy which revealed an atypical prepyloric ulcer. Biopsy revealed cancerous histology, similar to the initial biopsy. The patient continued to remain symptom free seven years after diagnosis.

The second case was a 64-year-old male who experienced progressive eating difficulty and weight loss. This patient was also diagnosed with poorly differentiated adenocarcinoma. Metastatic carcinoma was found in 17 of the 28 lymph nodes examined.

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Surgery was performed with no adjuvant chemotherapy following the anastomosis. The patient required several anastomotic dilations per year, as a benign stricture had developed. However, doctors found no evidence of recurrence for 16 years. Eventually, the patient refused further dilation procedures then died. The investigating author of this case report claims that a disease-free survival time of sixteen years, following late-stage discovery of adenocarcinoma, is unheard of and that five-year survival statistics are in the single digits. In subsequent interviews, the son of the patient attributed the good outcome to his father's use of noni juice.

2.1. Intervention Study

A human intervention study was conducted in 12 healthy volunteers (5 male: 7 female, age 20-40 yr) for 8 weeks, during which the volunteers each consumed 330 mL TNJ daily [22]. No other interventions were included. Vital signs, blood malondialdehyde (MDA), interleukin 2 (IL-2), and ex vivo natural killer-cell activity were measured before and after the TNJ regimen. After the 8 weeks, there was no change in vital signs. However, there was a significant decrease in mean MDA levels from 4.81 to 3.90 nmol/mL ($P < 0.01$), indicative of reduced lipid peroxidation/oxidative stress in the subjects. There were also significant increases ($P < 0.05$) in mean IL-2 concentration (from 52.5 to 69.2 pg/mL) and natural killer-cell activity (27.7 to 36%). The findings of this pilot study reveal the potential of TNJ to support immune function in a healthy population. Further, concurrent antioxidant and immunomodulation effects from noni juice were demonstrated.

2.1. Qualitative Survey

A qualitative survey of the uses of noni (also known as kura) was conducted in Fiji [23]. Consumers of noni were sought out from the general population in Fiji and interviewed. Researchers collected and analyzed data obtained from questionnaires filled in by the interviewers. There were 408 respondents in this survey. Questionnaire data included demographics of noni consumers, ailments for which noni was used, frequency of use, plant parts used, and methods of preparation. Among the conditions for which noni fruit was used were several that are associated with bacterial and viral infections. These conditions were gangrene, cough, bronchitis, pneumonia, tuberculosis, and sinus problems. The juice was also drunk as a tonic to enhance overall body strength. It is unclear from this survey whether the benefit from noni fruit is achieved through improved immune system performance against infection or whether perceived benefits are via symptomatic treatment only. However, against the background of existing research results, the findings are best explained by the demonstrated immunomodulating properties of noni fruit juice.

3. Animal Studies

Animal studies have further demonstrated the efficacy of noni juice in supporting immune system function, as well as has provided additional insight into possible mechanisms of action. These are summarized in Table 2.

Table 2. Animal studies of noni juice and immunomodulation.

Animals	No. in study	Noni juice dose	Duration	Outcome	Reference
Newborn Holstein bull calves	18	noni fruit puree at 30 mL/calf twice per day	14 days	increase phagocytic activity ex vivo	Shafer et al. 2008 [24]
Newborn Holstein bull calves	16	noni fruit puree at 30 mL/calf,	14 days	increased expression of IL-2 receptors on $CD4^+$ and $CD8^+$ T cells	Brooks et al. 2009 [26]

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		twicer per day			
Newborn Holstein bull calves	226	Noni fruit puree at 0, 15 or 30 mL every 12 hr	3 weeks supplementation, additional 3 weeks observation	54% reduction in total required medical treatments, 61% reduction in respiratory treatments and a 52% reduction in gastrointestinal treatments in noni fed calves	Brooks et al. 2013 [27]
C57BL/6J mice	10	100 ml/mice	15 days	increased IFN-γ, decreased IL-4	Palu et al. 2008 [28]
C57BL/6 mice	22	i.p. injection of 15 mg/mouse	5 days treatment, 50 days total observation	119% increase in survival time of LLC tumor bearing mice	Hirazumi et al. 1994 [30]
C57BL/6 mice	418	i.p. injection of 3 to 20 mg crude juice/mouse and 0.8 to 10.4 mg of noni extracts/kg	5 days treatment, 50 days total observation	significantly increased survival time of LLC tumor bearing mice, increased survival time with combination of noni juice precipitate and sub-optimal doses chemotherapeutic agents	Hirazumi and Furusawa 1999 [31]
DBA/2, C57BL/6, and BALB/c mice	434	i.p. injection of ethanol-insoluble precipitate from noni juice at 0.5 mg/mouse	50 days	increased survival time of S180 ascites tumor bearing mice	Furusawa et al. 2003 [32]
Swiss mice	45	6 g /kg	9 days	improved immune indices in gamma ray induced immunosuppressed mice	Nguyen et al. 2005 [36]
BALB/c mice	≥ 48	noni fruit extract at 200, 500, and 1000 mg/kg/day or deacetylasperulosidic acid 30 and 100 mg/kg/day	14 days	increased interleukin-2 (IL-2) concentrations and natural killer (NK) cell activity in immunosuppressed female mice	Murata et al. 2014 [37]
BALB/c mice	25	noni fruit extract 30, 90, 810 mg/kg/day	2 weeks of noni juice after 6 weeks of DMBA exposure and 3 days cigarette smoke (CS) exposure	Reversal of DMBA-induced decrease in $CD4^{+}TNF\alpha^{+}$, lowering of CS-elevated pro-inflammatory cytokine expression	Agustina et al. 2020 [41]

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Fisher (F344) rats	53	5 mL/kg of 5%, 10% and 20% of NFJ, twice per day	60 days	increased lymphocyte proliferation, IL-2 production, and ERK1/2 and Akt expression	Pratap et al. 2018 [42]
C57BL/6 mice	32	100 µL/mouse of noni juice or 1:10 and 1:100 dilutions	9 days	increase in total neutrophils and cytokine expression	de Sousa et al. 2017 [43]
Kunming mice	40	0.1, 0.2 and 0.4 mL/ mouse/day	21 days	dose-depended increase in CD3+ and CD4+ percentages, CD4+/CD8+ ratio and IL-2 and IL-4 concentrations	Yang et al. 2011 [44]
Chickens (commercial broiler chicks)	100	5 % in feed	day 1 from birth to six weeks	increased humoral immune response; trend of increased cell mediated response	Sunder et al. 2007 [50]

3.1. Bovine

The immunomodulating activity of noni fruit puree from French Polynesia was evaluated in newborn Holstein bull calves [24]. The calves were divided into two groups. The first was a control group that was fed milk replace every 12 hours for 14 days, in addition to water and other calf feed. The second group was also fed according to the same schedule, but also received 1 fl. oz. (30 mL) of noni fruit puree twice per day, added to the milk replacer, every 12 hours. Two mL of blood were collected from each calf on days 0 (for this trial, 36 to 48 hours of age), 3, 7, and 14. Cultures of *E. coli* and *Staphylococcus epidermis* were incubated with the blood samples, where percent bacterial kill was determined by the number of colony-forming units plated after incubation, compared to untreated cultures. By day 14, noni fed calves exhibited significantly greater gram-negative bacteria-killing (phagocytic), activity than control calves. An increased phagocytic activity has also been reported in rabbits fed an antioxidant-rich plant preparation [25]. Additionally, this preparation exhibited induction of humoral and cell-mediated immune responses, further suggesting the role of noni fruit antioxidant activity in the observed immunomodulation.

A second study, essentially following the same treatment schedule described above, measured mitogen-induced activation of $CD4^+$, $CD8^+$, and γδ T-cell receptor-positive T cells by measurement of CD25 (an IL-2 receptor) upregulation via 2-color flow cytometry [26]. Noni puree fed calves had increases in CD25 expression CD4+ and CD8+ T cells on day 3 of the study. Effects on CD8+ T cells were also observed over the course of this study.

A follow up study found that noni puree supplementation every 12 hours for the first three weeks of life in Holstein bull calves, on a farm with endemic salmonellosis, reduced required medical treatments by 54%, with a 61% reduction in respiratory treatments and a 52% reduction in gastrointestinal treatments [27]. These results reveal that the immunomodulation properties observed in the previous two trials correspond to clinically significant outcomes under real-world conditions.

3.2. Murine

The immunomodulating activity of commercial noni juice has been demonstrated in vivo [28]. Five C57BL/6 male mice were fed 100 mL TNJ/day for 15 days, while another 5 were provided water in lieu of TNJ (control group). On day 16, the splenocytes and peritoneal exudate cells were harvested. These cells were incubated for 16 h in the presence and absence of mitogen, followed by measurement of cytokine production by ELISA. The

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production of interleukin-4 (IL-4) was substantially inhibited by TNJ, compared to the control animals. However, interferon-gamma (IFN-γ) production was increased by TNJ treatment (IFN-γ is involved in macrophage activation). This increase in IFN-γ, corroborates the observed increase in phagocytic activity seen in newborn calves. An aberrant increase in IL-4 is involved in allergic responses and airway inflammation, such as in asthma. This finding is consistent with other observations of antioxidant compounds reducing the severity of asthma-like reactions [29].

Noni juice administered by intraperitoneal (i.p.) injection significantly increased the mean survival time of Lewis lung carcinoma (LLC) bearing C57BL/6 mice by up to 119%, compared to the controls [30]. There was no evidence for direct cytotoxic action from crude noni fruit juice or fruit extracts against LLC cells, even with significant increases in survival time with or without sub-optimal doses of standard chemotherapeutic agents [31]. Rather, modulation of the host immune system was proposed as the mechanism, since an extract from the juice was able to increase cytokine production in vitro. Further, concomitant treatment with immunosuppressive agents abolished the effect from noni.

Improvement in mean survival time was also observed in S180 sarcoma-bearing DBA/2, C57BL/6 and BALB/c mice treated i.p. with an extract from noni fruit [32]. The improvements in survival time in both LLC- and S180-bearing mice were abolished by treatment with immunosuppressive agents, thereby confirming the immunomodulatory action. Inhibition of cancer cell proliferation in vitro by other fruits has also been demonstrated [33], and antioxidant vitamins are reported to have promoted tumor immunity in humans [34]. Additionally, inhibition of Lewis lung carcinoma (LLC) by antioxidants has been reported previously [35].

The immune enhancing and protecting properties of noni fruit juice were investigated in gamma ray-induced immunosuppressed mice [36]. Four groups of 10 mice each received different treatments: blank controls (no treatment and no gamma ray exposure), a negative control group (no treatment, but exposed to gamma rays at 100 rad/d for 6 d), positive control group (received the same dose of radiation, but given 100 mg/kg levamisole, an immune stimulator, per oral for 9 d—3 prior to, and 6 days during, radiation exposure), and noni treatment group (same dose and treatment schedule as group 3, except that 6 g/kg noni fruit juice was administered in place of levamisole). The day after the 6 days of radiation exposure, the skin reaction against ovalbumin was assessed and then all animals were sacrificed. The relative thymus and spleen weights were measured, blood was collected, and additional indices of immunocompetence were measured and compared. These included total leukocyte and differential leukocyte counts, as well as rosette-forming cell and plaque-forming cell rates.

The relative spleen weight of the noni juice group was slightly greater than the negative controls ($P < 0.05$), but there was no difference in thymus weight. Total leukocytes of the noni group were 1.75 times greater than the negative controls ($P < 0.05$) and were even greater than in the positive controls. Noni-treated animals also had greater lymphocyte, monocyte, NK cell, and eosinophil populations than negative and positive controls. The neutrophil count of the noni group was also greater than that of the negative control group, but not greater than the positive controls. When compared to the negative controls, administration of noni juice increased humoral immunocompetence, as evidenced by a significant increase in rosette-forming cell ($P < 0.05$) and plaque-forming cell percentage ($P < 0.05$). These values were also greater for the noni juice group than for the levamisole (positive control) group. The skin reaction against ovalbumin of the noni group was 1.3 times that of the negative control group ($P < 0.05$), demonstrating a modest improvement in cell-mediated immunocompetence.

Similar to what had been reported in the 2-month human trial, serum interleukin-2 (IL-2) concentrations and natural killer (NK) cell activity were increased in immunosuppressed female BALB/c mice that were fed noni fruit juice extract, without any changes in T and B cell subpopulations [37]. Screening of compounds in further subfractions of the extract lead to the identity of the major phytochemical constituent of noni fruit, deacetylasperulosidic acid, as being the most active in a delayed-type hypersensitivity model.

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Immunosuppressed mice were also fed pure deacetylasperulosidic acid daily for two weeks. This also significantly increased plasma IL-2 concentrations and NK activity. This demonstration of the prominent role of iridoids in the immune modulating properties of noni fruit is not surprising. Aucubin, which only differs from deacetylasperulosidic acid by the absence of a carboxyl group, stimulates IFN-γ secretion from human peripheral blood mononuclear cells [38]. A combination of two iridoid glycosides, picroliv, augmented antigen-specific human T cell response, enhanced macrophage migration index and phagocytosis of pathogenic bacteria, as well as protected hamsters against *Leishmania donovani* infection [39,40].

The chemoprotective properties of noni juice were evaluated in five weeks old male Balb/C mice. 7,12-Dimethylbenz(a)anthracene (DMBA) was administrated for six weeks followed by 3 days of cigarette smoke (CS) exposure [41]. The mice were then treated with varying doses of noni juice for two weeks. Control animals received no noni juice. In this study, noni juice increased CD4+TNFα+ cells after DMBA-CS exposure, while also lowering CS-elevated pro-inflammatory cytokine expression. These results suggest that noni juice functions as a T cell regulator.

Old (16–17 month) male F344 rats were fed 5 mL/kg body weight of either 0% (saline only), 5%, 10% and 20% noni juice twice per day for 60 days [42]. Afterward, lymphocyte proliferation, cytokine production and expression of intracellular markers were compared among the different groups, as well as compared to young untreated controls. It was revealed that noni juice increased concanavalin A-induced lymphocyte proliferation, IL-2 production, and phospho-extracellular signal-regulated kinase ½ (p-ERK1/2) expression. Additionally, phospho-protein kinase B (p-Akt)/total Akt expression was significantly greater in noni juice-treated old rats, as compared to the saline-treated controls. Noni juice ingestion also decreased phospho-nuclear factor-κB (p-NF-κB) expression. It also restored age-related declines in the activity of superoxide dismutase (SOD), an antioxidant enzyme involved in the reduction of destructive superoxide anion radical (SAR) within the body. The results of this study indicate that noni juice has the potential to reverse age-related declines in lymphocyte proliferation, IL-2 production, and SOD activity via p-ERK1/2, Akt and NF-κB signaling. As such, it may upregulate beneficial cell-mediated immune responses while limiting pro-inflammatory responses in the lymph nodes.

Male C57BL/6 mice were fed 100 μl of single-strength noni juice or dilutions (1:10 and 1:100) thereof for 9 days [43]. Leukocyte count was increased in the mice fed single-strength noni juice, when compared to saline-treated controls. This increase appears to be due to the statistically significant increase in total neutrophils. This observation seems to be consistent with the increased phagocytic activity of blood samples obtained from noni fed calves, as discussed above.

Four groups of mice (n=10) were fed different concentrations of TNJ twice per day for 21 days [44]. Flow cytometry was used to measure CD3+, CD4+, CD8+ T lymphocyte populations and to determine CD4+/CD8+ ratios. Serum IL-2 and IL-4 were measured by enzyme-linked immunosorbent assay. There was a dose-depended increase in CD3+ and CD4+ percentages as well as a dose-dependent increase in CD4+/CD8+ ratio. There were concomitant dose-dependent increases in IL-2 and IL-4 concentrations, both of which are associated with CD4+ function. CD4+ cells are T helper cells with a central regulating role in the immune system. CD8+ cells (suppressor T cells) down regulate immune function [45-47]. The CD4+/CD8+ ratio is commonly used to assess immune system function. In a properly functioning immune system, this ratio is greater than one, as CD4+ cell count should be greater than CD8+. As reductions in the CD4+/CD8+ ratio have been associated with immune deficiency, the dose-dependent increase in this ratio indicates an increase immune system function that occurs with noni juice ingestion [48,49].

3.1. Avian

The immunomodulating effect of noni juice was evaluated for six weeks in 100 d-old commercial broiler chicks [50]. The chicks were divided equally into four groups. One group served as a no-treatment control. Another group served as controls challenged with

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infectious bursal disease virus (IBDV) at week 3. The other two groups were provided water with noni juice (5%). Only one of the noni juice groups was challenged with IBDV. Body weight gains were recorded for each group, as well as feed efficiency measurements. The humoral immune response of chicks was evaluated by the hemagglutination (HA) test against goat red blood cells. Cell-mediated immunity was evaluated by measurements of phytohemagglutinin induced inflammation of the foot. Following IBDV challenge, survival rates were compared. The noni feed group experienced more weight gain and better feed efficiency than the controls. The antibody response, as measured by HA titer, was significantly greater in the noni fed chicks ($P < 0.05$). There was a trend of improved cell-mediated immunity in the noni group, as measured by phytohemagglutinin-induced inflammation. Mortality in the noni group was much less (6.6%) than the controls (25%) following IBDV challenge

4. Human Antioxidant Studies

Oxidative processes are involved immune system activity. Dietary antioxidants, such as those from plant-based foods, have also been previously reported to have a positive impact on immunity [51]. Studies reporting the antioxidant activity of noni juice in human subjects are summarized in Table 3 and discussed below.

Table 3. Human studies of noni juice (TNJ) antioxidant properties.

Study population	No. in study	Noni juice dose	Duration	Outcome	Reference
Healthy adults	12	330 ml/day	8 weeks	Reduced plasma malondialdehyde (MDA) levels	Ma et al. 2008 [22]
Heavy cigarette smokers	285	29.5 mL and 118 mL/day	One month	Reduced plasma superoxide anion radicals and plasma lipid hydroperoxides	Wang et al. 2009 [55]
Heavy cigarette smokers	245	29.5 mL and 118 mL/day	One month	Reduced lipid peroxidation-derived DNA adducts in peripheral blood lymphocytes	Wang et al. 2013 [57]
Athletes	40	200 mL/day	Three weeks	Reduced lipid peroxidation (blood chemiluminescence)	Palu al. 2008 [64]

As described above, a human intervention study was conducted with 12 healthy volunteers for 8 weeks, during which the volunteers consumed 330 mL TNJ daily [22]. A concurrent increase in IL-2 concentrations and natural killer-cell activity accompanied a significant decrease in blood MDA levels ($P < 0.01$), thereby demonstrating a connection between the antioxidant properties of TNJ and its immunomodulating properties.

The antioxidant properties of TNJ were also evaluated in cigarette smokers. Cigarette smoke is well known to contain many oxidant molecules capable of causing various forms of oxidative damage [52]. Further, it has been demonstrated that cigarette smoking increases peroxidation products in plasma [53,54]. Therefore, current heavy smokers, those who smoked more than 20 cigarettes per day for at least one year, were chosen for this double-blinded, placebo-controlled clinical trial [55]. Two hundred and eighty-five volunteers, between the ages of 18 and 65, were randomly divided into three groups, a 29.5 mL TNJ daily group ($n = 121$), a 118 mL TNJ daily group ($n = 122$) and a placebo group ($n = 42$). The placebo in this trial consisted of a mixture of grape and blueberry juice that was flavored with a natural cheese flavor. The unique flavor of noni fruit is due to caproic and

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caprylic acids, fatty acids that are characteristic of cheese [56]. Therefore, the natural cheese flavor was included in the placebo to mimic the flavor of TNJ. The volunteers drank their assigned TNJ dose or placebo for 30 days. Ten mL of blood was drawn from each volunteer before and after the trial. Plasma was from the blood samples were and subjected to SAR and lipid hydroperoxides (LOOH) analysis, with pre- and post-plasma values being compared statistically.

There were no significant changes in plasma SAR and LOOH levels in the placebo group throughout the study. On the other hand, the mean plasma SAR level of the TNJ groups decreased by at least 27% ($P < 0.001$) after 30 days of TNJ consumption. At the end of the trial, the mean plasma LOOH concentrations of both TNJ groups were 32% below that of the placebo group ($P = 0.001$). The results of this trial demonstrate that antioxidant activity of noni juice is observable in human subjects and is not limited only to animal experiments and in vitro studies.

Data which further demonstrate antioxidant activity in humans are found in the investigation of the change in LOOH-DNA adducts in the lymphocytes of heavy smokers [57]. Lipid hydroperoxides and their decomposition product malondialdehyde (MDA) are highly reactive end products of lipid peroxidation that have been widely used in research to measure levels of oxidative stress in vivo. In addition to other oxidation products, cigarette smoke exposure has been demonstrated to increase plasma MDA [58,59]. Additionally, increases in DNA adducts, such as MDA-DNA adducts, have been associated with cigarette smoke in both animals and humans [60-62]. DNA adducts levels in peripheral blood lymphocytes (PBLs) are highly correlated with smoking [63]. As a result, MDA-DNA adduct in PBLs are good biomarkers for oxidative stress induced by tobacco smoke and can be used to clinically investigate the antioxidant activity of TNJ in humans. Therefore, a double-blinded, placebo-controlled clinical trial involving 245 heavy smokers was conducted to evaluate the effect of TNJ consumption on MDA-DNA adduct levels in PBLs. Participants were randomly assigned to a placebo group (n = 42), a 29.5 mL TNJ group (n = 118), or a 118 mL TNJ group (n = 85). For 30 days, participants consumed their assigned doses of placebo or TNJ. Blood samples were drawn from each participant at enrollment and at completion of the 30-day period. DNA was isolated from PBLs that had been isolated from whole blood. MDA-DNA adducts were determined by ^{32}P-postlabelling after radioactive phosphorous had been incorporated into sites where adducts had been removed and the DNA repaired. The ^{32}P-labeled DNA was purified and separated by three-dimensional TLC chromatography, followed by detection by autoradiography and scintillation counting.

The mean MDA-DNA adduct levels in the placebo group of this clinical trial did not undergo any significant change after 30 days. However, noni juice ingestion decreased MDA-DNA adduct levels in PBLs by approximately half. The mean MDA-DNA adduct levels of the TNJ groups decreased significantly, by 44.6 to 57.4%. There was no difference of the antioxidant effectiveness between females and males. The results of this study indicate that both TNJ doses were effective in mitigating oxidative damage leading up to the formation of MDA-DNA adducts. It provides further evidence of the antioxidant activity of TNJ in humans.

A study of the antioxidant properties of noni juice was performed under conditions of physical exertion [64]. This was a controlled clinical test of the effects of TNJ on exercise performance in 40 highly trained athletes (middle- and long-distance runners; 1500 to 10000 m) between the ages of 18 and 27 years old. Athletes were divided into 2 groups of 20 each (16 males and 4 females/group). Each group was assigned to drink either 100 mL TNJ, or placebo (blackberry juice), twice/day, for a total of 200 mL/day. This dose schedule was followed for 21 days.

At the beginning of the trial, the athletes were given medical examinations, and blood and urine samples were collected for analysis. Exercise endurance-regeneration rate, glucose, protein and urea content of the blood, as well as spontaneous chemiluminescence in the urine, were measured before the experiment, and on day 10, 21, and 5 days after cessation of the dose schedule. Exercise endurance was measured by athletes running on a

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treadmill where the exercise load is increased every min. The time to exhaustion is recorded for each treadmill run. Exercise endurance-regeneration rate is determined by biochemical analysis of blood glucose, total protein, and lactate, etc. Blood analysis did not reveal any significant difference between the TNJ and placebo groups. Blood glucose, protein, and urea were unchanged after the 21-day trial. The exception is that blood lactate increased in the placebo group over the 21 days, but not in the TNJ group. The discrepancy in blood lactate in the two groups indicates that consumption of TNJ improved efficiency in performing the physical work, as energy consumption did not increase.

Exercise endurance in the TNJ group was increased significantly, while no such effect was observed in the placebo group. The mean time to exhaustion had increased for athletes drinking TNJ by 21.8% ($P < 0.05$) by day 21, an average increase of 2.6 min in the treadmill run with an increasing load. As blood analysis revealed that the improved endurance was not related to increased energy consumption, another mechanism was responsible for this. One identified mechanism was antioxidant activity.

Chemiluminescence is the phenomenon of light emission (or photon emission) from excited lipid oxidation products in biological matrices [65]. This method has been available for decades but has been used more extensively used within the past decade for clinical investigations [66]; including the measurement of chemiluminescence as an indicator of oxidative stress [67]. Chemiluminescence has been previously used as an indicator of lipid peroxidation associated with heavy exercise [68,69]. To assess the antioxidant effect of TNJ, chemiluminescence was measured for all participants at the start of the trial and after 21 d, as well as 5 days after cessation of the dose schedule. The amount of chemiluminescence in the TNJ group decreased by approximately 25% ($P < 0.05$), whereas no decrease was evident in the placebo group. These results reveal significant antioxidant action from TNJ.

Strenuous and long-duration exercise can produce oxidative stress [70]. Further, free radical-induced damage in muscle is a factor in muscle fatigue [71], and antioxidant intervention has been demonstrated to inhibit muscle fatigue [72]. Therefore, the antioxidant activity of TNJ is responsible for the improved endurance in the athletes evaluated in this clinical trial.

5. Conclusions

The ability of noni juice to influence the function of the immune system has been demonstrated in both human and animal studies. Such effects have been seen with as little as 0.8 mL/kg via the oral route. The results from the several human and animal studies demonstrate good agreement between effects. In humans, increased IL-2 and natural killer-cells activities have been observed. The role of IL-2 in the body's response to microbial infection is well known. The increase in IL-2 seen in humans is likely to have occurred in the Holstein calves and was involved in the increased phagocytic activity against *E coli*. The role of natural killer cells in destroying tumors and cells infected by viruses is also well known. Therefore, the increased natural killer-cell activity is a likely a major contributor to the improved outcome and survival described in the case report, as well as seen in LLC and S180 tumor bearing mice. Increase IFN-γ production was seen in mice fed TNJ. IFN-γ promotes natural killer-cell activity and macrophage lysosomal activity (a function of phagocytosis), activities both seen in the human and bovine studies.

The interplay between antioxidant and immunomodulating activities has been established in healthy human volunteers. The role of antioxidants in healthy immune function has been well described. The effect of noni juice on the human immune system is supported by evidence of its antioxidant action in humans.

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