REVIEW ARTICLE

Immunomodulatory Functions of *Moringa oleifera* (Lam.)

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ABSTRACT

Consumption of herbs as dietary supplements leads to beneficial effects on human health by maintaining a competent physiological system that balances homeostasis. It has been widely known that herbal-based products preserve good health by preventing or ameliorating chronic diseases such as metabolic, cardiovascular, inflammatory, neurodegenerative and neoplastic diseases. Although many herbs are known to boost the immune response via various mechanisms, only a few herbs are known traditionally to have a holistic beneficiary effect on the entire immune system, ranging from enhancing the stem cells pool to improving the quality of functional immune cells. In this review, significant scientific discoveries of *Moringa oleifera*'s immunomodulatory functions discussed in both *in vitro* and i*n vivo* models. Besides its immunomodulatory actions, other reported biological functions of Moringa oleifera are also highlighted, along with relevant phytochemicals.

Keywords: *Moringa oleifera*, immune system, Immunomodulation, Phytoimmunotherapy, Immunonutrition, Immunostimulants

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INTRODUCTION

The immune system, the guardian of the body's defence network, provides the body complete protection from invading pathogens. The immune system executes its functions in many levels, ranging from cells to soluble factors classified as cell and humoral mediated immunity. However, such immune responses can be severely affected in immunodeficiency disorders, which could be manifestation of decreased quantity of immune cells or compromised quality of immune responses. In such circumstances, the immune system loses the capability to protect the body from the protruding infectious agents (1). The ill-state immune system prejudices to nosocomial and opportunistic infections; hence, surges the risks and chances of developing benign and malignant tumours resulting from decreased cancer immunosurveillance (2). The changes in immune response either in the form of immunoboosting or immunosuppression is collectively termed as immunomodulation. Imunomodulation is an immune homeostatis process that could occurs at transcient period to balance out the ongoing immune response to achieve an immune equilibirium. The preferred action of immunomodulation can be induced with food consumption or supplementation of plants materials with established immunomodulatory properties (3, 4). This is often referred to as "Phytoimmunotherapy", which is considered a new approach for treating cancers (3, 5).

Moringa oleifera Lam.

One of the local herbs that could exert this wholesome 'wonder' activity is *Moringa oleifera* or locally known as 'kacang kelor' in Malay and 'Murunggai' in Tamil. *Moringa oleifera*, a family Moringaceae, is a small-medium sized tree, 10–15m high, widely cultivated in East and Southeast Asia, Polynesia and the West Indies. Indians have been using *Moringa oleifera* as a regular component of conventional eatables in Ayurveda and Siddha medicine for nearly 2000 years (6). Almost all parts of *Moringa oleifera*, namely, the leaves, flowers, fruits, resin (gum) and the tree's bark, have been fully utilised in India for gastronomical and therapeutical purposes. The famous Tamil proverb often cites that 'having one *Moringa oleifera* tree in your backyard will make you raise the kids easily' reflected by the outstanding amounts of the nutritional component in *Moringa oleifera* (7). In addition, the pods and leaves of Moringa contain a high amount of Ca, Mg, K, Mn, P, Zn, Na, Cu, and Fe (8). The *Moringa oleifera* tree is a

deciduous and fast-growing tree that can grow to as high as 15 meters with broad branches, alternate, pinnate feathery leaves, white or cream-coloured flowers and rounded green fruits (9) (Figure 1).

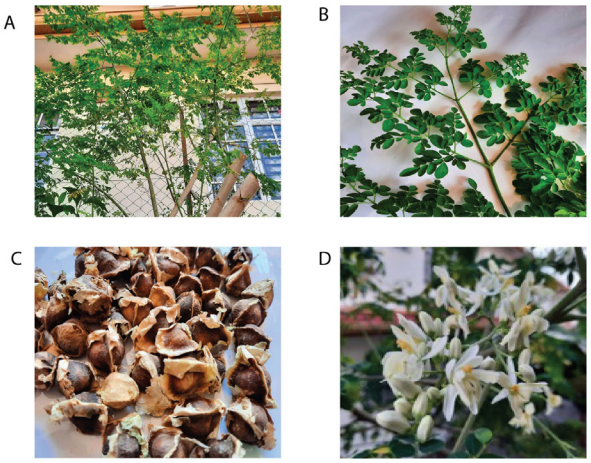

Figure 1: Parts of a locally available *Moringa oleifera* tree

PHYTO BIOCHEMISTRY OF *Moringa oleifera*

The most commonly found phytochemicals can be structurally classified into three groups: (10) compounds with a 2-phenyl-1,4-benzopyrone backbone often referred to as Flavonoids (11) compounds with β-thioglucoside N- hydroxysulfate motif referred to as Glucosinolates, and (12) phenolic acids with benzoic and cinnamic acids linked by hydroxyl groups) (13). In *Moringa oleifera* leaves, studies have reportedly found glucosinolates, and this compound can be hydrolysed by the enzyme thioglucoside glucohydrolase (Myrosinase) to form thiocyanates, isothiocyanates and nitriles (14, 15). For this reason, many glucosinolates and isothiocyanates similar to those found in crucifers like 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate benzyl isothiocyanate have been found in *Moringa oleifera* leaves. Similarly, different nitriles glycosides such as niazirin, niaziridin and niazirinin as well as their derivatives like niaziminin A, and niaziminin B have been found (16, 17).

The most abundant phytochemicals in leaves of *Moringa oleifera* are glycosylated flavonoids like quercetin-3-O-glucoside (isoquercetin), quercetin-3-O-rutinoside (rutin) and kaempferol-3-O-glucoside (astragalin), which can readily form the aglycone compounds quercetin and kaempferol (18). In addition, chlorogenic acid, ferulic acid, gallic acid, and ellagic acid have been present in *Moringa oleifera* leaves extracts by fractional studies and other compounds such as β-sitosterol, hyperoside, oleanolic acid, rutoside, and terpenoids (19).

Phytochemical studies on *Moringa oleifera* revealed major polyphenols such as quercetin glucosides, rutin, kaempferol glycosides and chlorogenic acid by HPLC analysis (20, 21). Faizi et al. (1994) reported the isolation of two nitrile glycosides from the ethanolic extracts of *Moringa oleifera* leaves, niazirin and niazirinin and three mustard oil glycosides (22). Singh et al. (2009) reported the presence of gallic acid, chlorogenic acid, ellagic acid, ferulic acid, kaempferol, quercetin and vanillin from the aqueous extracts of leaves, fruits and seeds (23).

BIOLOGICAL ACTIVITIES OF *Moringa oleifera*

The biological/pharmacological properties of *Moringa oleifera* have been extensively explored by many studies in series of *in vitro* and *in vivo* research involving diabetes mellitus, liver function, inflammation, obesity and cancer (24, 25). Extracts from the leaves have been obtained using different solvents such as ethanol, water, n-hexane, chloroform and methanol and have been reported to exert hypoglycaemic, hypocholesterolemic, antitumour, antioxidant, antiarthritic, antinociceptive, immunomodulatory, hepatoprotective, anti-inflammatory, antimicrobial and radioprotective properties (26-28). Additionally, studies have reported antiulcer activity of aqueous and methanol extracts of *Moringa oleifera* leaves in aspirin, indomethacin and serotonin-induced rats through prevention of the formation of gastric lesions and modulation of the secretion of 5-hydroxytryptamine (5-HT) via the 5-HT3 receptors of enterochromaffin (EC) cells in the gastrointestinal tract (29, 30). Furthermore, the leaves powder of *Moringa oleifera* has been reported to be rich in vitamin A (31). Hence, it may be therapeutically helpful in treating ocular diseases as consumption of the leaves improves vitamin A nutrition and slows the development of cataracts (32).

The ability of *Moringa oleifera* leaves aqueous extract to regulate levels of thyroid hormones and its potentials in the treatment of hyperthyroidism have been identified by previous studies (33). Also, studies have shown that methanolic extracts of *Moringa oleifera* leaves significantly protected mice bone marrow cells chromosomes against radiation (34, 35). These properties can be ascribed to the nutritional and phytochemical constituents of the leaves as it possesses some high amounts of vitamins, minerals and amino acids such as cysteine, lysine, methionine and tryptophan, as well as phytochemicals like flavonoids which are known to display high bioactivity (Table I).

EFFICACY AND SAFETY OF *Moringa oleifera*

As highlighted in the previous sections, many *in vitro* and *in vivo* studies have described the ability of *Moringa oleifera* leaves extracts to produce a wide range of desired physiological and pharmacological activity effectively. However, very few of these studies have ventured into extract standardisation and utilised the standardise extracts in the experiments conducted; for

Table I: Pharmacological activities of *Moringa oleifera* leaves

Extraction Method	Experimental model	Effect/ Activity	References
Methanolic	Rat model	Hepatoprotective	(25)
Ethanolic	AML and Hepatocarcinoma cell line	Anticancer	(45)
Aqueous Ethanolic	MRSA and Klebsiella Spp	Antimicrobial	(46)
Aqueous Ethanolic	HeLa Cell line	Antitumour	(47)
Aqueous	HeLa cell line & Lymphocytes	Anticancer	(48)
Ethanolic	Rat model	Antiarthritic/ anti-nociceptive	(28)
Methanolic	HepG2, Caco-2 and MCF-7 cancer cell line	Antitumour	(26)
Aqueous	Human tumour (KB) cell line	Antitumour	(27)
Aqueous	Panc-1 and COLO-357 cell lines	Antitumour	(49)
Methanolic	Swiss albino mice	Antitumour	(35)
Aqueous Ethanolic	Swiss albino mice	Radioprotective	(36)
Ethanolic	Swiss albino mice	Radioprotective	(50)
n-hexane	Rat model	Hepatoprotective Antihyperlipidemic Anti-inflammatory	(51)
Aqueous Ethanolic	Swiss albino mice/ mice erythrocytes	Antioxidant	(29)

this reason, it is challenging to conduct a comparative analysis between the findings of one study with another (36). Similarly, the extraction procedures used in most studies are not bioactivity-based, and as such, the relationship between extraction of the chemical constituents and the reported pharmacological activity remains ambiguous (37). It is also unclear whether the different components of the extract preparations interact via an inhibitory, additive or synergistic mechanism to yield the pharmacological outcome.

Although most studies on *Moringa oleifera* employed *in vitro* or experimental animal models, where solvents such as water, ethanol, methanol, hydroalcoholic were used for extraction, nearly all the published studies involving humans have used powdered leaves preparations (36). The general toxicity of *Moringa oleifera* leaves has been assessed in many *in vitro*, animal and human studies. Peripheral blood mononuclear cells (PBMCs) isolated from healthy human donors were treated *in vitro* with different concentrations of *Moringa oleifera* extract, and a cytotoxicity assay was conducted. The results obtained showed that toxicity occurred at 20 mg/mL, an unachievable concentration by oral administration. The same study conducted an acute toxicity analysis in rats and reported genotoxic effects at a dose of 3000 mg/kg for 14 days while 1000 mg/kg was observed to be safe (38). Adedapo et al. orally administered 400, 800, 1600, and 2000 mg/kg body weight aqueous extract of *Moringa oleifera* leaves to rats daily for 21days and inferred that 2000 mg/kg dose of *Moringa oleifera* leaves aqueous extract was safe (39). Mice 50% lethal dose (LD_{50}) of *Moringa oleifera* leaves aqueous extract has been reported to be approximately 1585 mg/kg in an acute/ sub-chronic study (40), while another acute toxicity study on orally administered *Moringa oleifera* ethanol extract in mice, estimated the LD_{50} > 6.4g/kg (41).

In humans, powdered *Moringa oleifera* leaves have been used in trials to treat hyperglycemia (42) and dyslipidemia (43). These studies used a maximum daily dose of 8.0 g for 40 days and a maximum single dose of 50 g; however, although no adverse side effects were reported, it is essential to highlight that no study has used leaves extract of *Moringa oleifera* on human subjects.

Recently, interest in exploring the immunomodulatory effect of *Moringa oleifera* on the immune system becomes increasingly evident in scientific articles. For example, the anti-respiratory burst and anti-chemotactic properties of ethanol extract of *Moringa oleifera*'s Quercetin 3-O-glucoside, crypto-chlorogenic acid, and kaempferol 3-O-glucoside on neutrophils were reported. However, investigation of other functional properties of neutrophils and other immune cells is still lacking (44). The immune system can be manipulated with specific dietary intake; hence, it's becoming an essential tool to prevent immune-based chronic disease and can serve as supplementary therapies for several diseases, including malignancies. The robust expansion of functional food supplements from the plant products has created new niche "immunonutrition" (45). Hence, *Moringa oleifera* leaves has the full potential to be developed as an immunomodulator that could cater for the general population, high-risk individuals and immunosuppressed patients.

IMMUNOMODULATION

The functions of the immune system in ensuring protection against infectious diseases and cancers have been elaborately explored by many immunologists, oncologists, and other scientists, as they identify the immune system as a target for developing therapeutic interventions. However, the immune system is highly regulated. As a result, there is a paradigm shift in the focus of many researchers on understanding the immune system's regulatory mechanisms and how these mechanisms can be modulated to target diseases and

malignancies (46).

Also, the last two decades have witnessed a surge in infectious agents, leading to the capitulation of antibiotics (47, 48), hence the development of selective individualised immunomodulation-targeting therapies with consideration to pharmacogenomics proven to be more successful (49, 50). Furthermore, oncologists have recorded dismal benefits from single-agent chemotherapeutic and radiation treatments, and as a result, they have developed the concept of exploiting the antitumour defence mechanism of the host in anticancer therapies (51). Although immunomodulation in therapeutics may significantly promote the development of immunity, excellent care and caution should be taken to prevent undesired immune reactions (52).

The concept of immunomodulation is the selective regulatory adjustment of the immune system, enhancing, inducing or suppressing an immune response by the action of an immunomodulatory agent (46). Therefore, agents that enhance the host's resistance against infections, cancers and autoimmunity, by mobilising the innate and adaptive immunity are referred to as immunostimulants. These agents can act as immunotherapeutic interventions of individuals with primary and secondary immune deficiencies, i.e. aged and immunocompromised persons, as well as serve as prophylactic immune potentiators that improve the immune response in healthy individuals (53, 54).

Immunosuppressants are agents used to prevent or diminish immune response in pathological situations like immune-related infections, autoimmune diseases, immediate or delayed-type hypersensitivity, graft versus host disease and graft rejection (55). In organ transplantation, the use of immunosuppressants is aimed at slightly shutting off the immune response to the allograft while still maintaining enough immunity to resist opportunistic infections and malignancy (56). The third group of immunomodulatory agents are the immune adjuvants; although they do not possess any specific antigenic property, they can stimulate the immune system or increase the response to a vaccine (57). Based on the therapeutic target, immune adjuvants can act by controlling the availability of an antigen, facilitating the binding of an antigen to immune cells, enhancing phagocytosis, ensuring that immune response is specific to a selected antigen, as well as serve as a warning signal for the immune system (57, 58) (Figure 2).

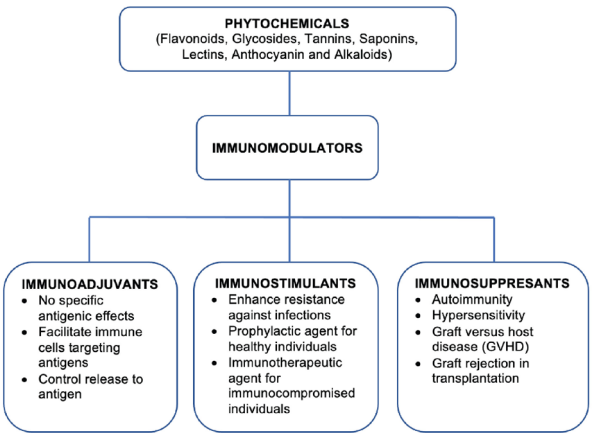

Figure 2: Potentials of phytochemicals as immunomodulatory agents

PHYTOCHEMICALS AS IMMUNOMODULATORY AGENTS

As naturally occurring compounds with immense bioactive potentials, phytochemicals such as flavonoids, phenols, polysaccharides, tannins, lectins, glycosides, coumarins, terpenes and terpenoids have been extensively explored by many types of research for different therapeutic activities (59). Although these phytochemicals are synthesised by plants, there are not exclusively necessary for plant survival. However, recent studies suggest that these "secondary metabolites" may play significant roles in plant disease resistance (60, 61). However, in animals, phytochemicals have, in addition to antioxidant, antidiabetic, anti-inflammatory and anticancer properties, displayed impressive immunomodulatory prowess. For example, the ability to induce the proliferation of natural killer cell in animal models as well as improve their cytotoxic and antitumour activity has been observed in plant lectins from *Viscum album* (62). Generally, through interactions with cell surface glycoproteins, lectins can alter the gene expression of cytokines and other proteins (63). The flavonoids quercetin-3-O-rutinoside and kaempferol-3-O-rutinoside have shown immunostimulatory effects on neutrophils (64). Similarly, luteolin from Platango major has exhibited the ability to enhance lymphocyte proliferation and secretion of Interferon gamma (IFN-γ) (65). Alkaloids like piperine have been reported to increase absolute leukocyte count, increase antibody production, and bone marrow cellularity, while berberine reduces plasma levels of tumour necrosis factor alpha (TNF-α), IFN-γ and nitric oxide (NO) (65, 66). Likewise, phenolics such as chlorogenic acid have been shown to enhance lymphocyte proliferation and stimulate IFN-γ production while gallic acid inhibits mast cell degeneration and stimulates B-cell proliferation (65, 67).

Additionally, chebulagic acid, a tannin, has been reported to induce the downregulation of TNF-α and interleukin 6 (IL-6), while andrographolide, which is a terpenoid from *Andrographis paniculata*, enhances interleukin 2 (IL-2) expression and inhibits NO production in endotoxin, stimulated macrophages (68, 69).

IMMUNOMODULATORY EFFECTS *Moringa oleifera IN VITRO* MODELS

A concentrate rich in isothiocyanates from *M. oleifera*

leaves extract could inhibit both the gene and protein expression of interleukin IL-1β and IL-6 in lipopolysaccharide (LPS) stimulated RAW 264.7 cells (70). Similarly, the ethyl acetate fraction of M. oleifera, rich in phenolics, was reported to inhibit macrophage activation by LPS or cigarette smoke. The ethyl acetate extract was able to decrease the expression of Rel A (p65), which plays a prominent role in the activation of the nuclear factor kappa β (NF-kβ) pro-inflammatory pathway. Furthermore, reduced gene and protein expression of pro-inflammatory cytokines (IL-6, IL-8 and TNF-α) was observed (71). In keeping with the above finding, Arulselvan and colleagues showed that the anti-inflammatory effects of the ethyl acetate fraction of *M. oleifera* result from blockade of Iκβα dissociation from the Iκβα-p65-p50 complex. This prevents the translocation of NF-kβ into the nucleus, resulting in decreased expression of pro-inflammatory cytokine release (72). A similar finding was reported in some compound isolated from M. oleifera. For instance, isothiocyanates enriched seed extract (MIC-1) of *M. oleifera* was reported to exert different physiological response in LPS stimulated-macrophages (RAW 264.7); however, all leading to downregulation of the immune response. At a dose of 1 mM, it reduced the production of NO. At 5 mM its inhibit the gene expression of LPS-inducible nitric oxide synthase (iNOS) and cytokines (IL-1β and IL-6), an increase of the concentration to 10 mM result to increased expression of anti-inflammatory transcription factor (nuclear factor erythriod derived-2-like 2 (Nrf2) and its targets genes NAD(P)H: quinone oxidoreductase 1 (NQO1), glutathione S-transferase pi 1 (GSTP1), and heme oxygenase (HO1) (73). Similarly, a polysaccharide isolated from the root of *M. oleifera* (MRP-1) also dampened the release of NO and TNF-α in murine macrophage RAW-264.7 (74). Additionally, a recent study by Leutragoon and colleague in 2020 revealed further the anti-inflammatory effect of ethyl acetate fraction of *Moringa oleifera* leaves extract against LPS-stimulated human monocytes and macrophages. The extract and the fractions were able to inhibit the phosphorylation of Iκβ, phosphorylation of Rel A (p65) and nuclear translocation of NF-κβ, resulting in decreased mRNA expression of IL-1, IL-6, TNF-α, iNOS and cyclooxygenase (COX-2). Thus, leading to decrease synthesis and release of pro-inflammatory mediators of the immune cells (76).

On the other hand, other polysaccharides (MOP-2 and MOP-3) extracted from the leaves of *M. oleifera* were demonstrated to enhance the proliferation and pinocytic activity of unstimulated macrophage (RAW 264.7) as well as increase secretion of reactive oxygen species (ROS). These polysaccharides were able to promote the expression of iNOS, IL-6 and TNF-α mRNA, with a resultant increase in the release of NO, IL-6 and TNF-α molecules (76, 77). *Moringa oleifera* seed resistant protein (MSRP) stimulates the proliferation of murine splenocyte and NO production from macrophages and increases the proliferation of lymphocytes (78). *Moringa oleifera* water-soluble lectins could induce the release of TNF-α, IL-2, IL-6, IL-10 and nitric oxide (NO) in PBMCs. In addition, the water-soluble lectins were able to promote the activation of cytotoxic CD8+ T cells (79). In Chagas disease, an endemic lethal disease in Latin America, caused by *Trypanosoma cruzi* can infect the peripheral immune cells (PBMCs), leading to parasitemia and progression to Chagas diseases (80). Studies using trypsin inhibitor isolated from the flower of *M. oleifera* showed interesting result on *T. cruzi* infected PBMCs. The trypsin inhibitor at a dose of (43.5 or 87.0 mg/ml) increased the release of TNF-α and INF-γ from *T. cruzi* infected PBMCs, which will attract cytotoxic T-cells (CD8+) to the infected cells and their phagocytosis. In addition, there is an increase in anti-inflammatory cytokine release (IL-10), yet demonstrating the potential of *Moringa oleifera* plant in controlling inflammation (80).

These findings clearly point to an interesting nature of *Moringa oleifera* plants extracts in controlling inflammation in stimulated immune cells demonstrating potential in autoimmune diseases, transplantation therapy, acute and delayed hypersensitivity reactions. On the other hand, the immunostimulatory effect of *Moringa oleifera* plant extracts and fractions can be utilized in immunocompromised conditions such as acquired immune deficiency syndrome and human immune virus (HIV/AIDS) (72, 81-84). In summary, *Moringa oleifera* modulatory effects on the immune cells are context-dependent, inhibiting stimulated cells and activating resting or un-stimulatory cells.

IMMUNOMODULATORY EFFECTS *Moringa oleifera IN VIVO* MODELS

In the murine experimental model, methanolic extract of *Moringa oleifera* adminstration improve adhesion and phagocytic activity of neutrophil. Moreover, it enhances the secretion of immunoglobulins (antibodies) of interest in the rescue of neutropenia induced by cyclophosphamide (85). In keeping with the above finding, administering 1000 mg/kg body weight of methanolic extract of *Moringa oleifera* to albino Wistar rats increases their white blood cell count, lymphocyte and neutrophil count (86). Furthermore, *Moringa oleifera* methanolic extract also promotes the proliferation of T lymphocytes and splenocytes in rat immunised with *Salmonella typhimurium* "O" antigen (87).

In addition, *Moringa oleifera* extract exerts protection in rat intestine against *Hymenolepiasis nana* infections through decreased TGF-β, IFN-γ and mucosal mast cells with a corresponding increase in goblet cell count, T-helper cell type 2 (Th 2), cytokines (IL-4, 5, and 9) and secretory immunoglobulins (IgA) level (88). Furthermore, *Moringa oleifera* significantly counteracts the decrease in WBC, neutrophil, lymphocyte. Moreover, Improvement

in phagocytic function, lysozyme activity, nitric oxide, and total IgM and IgG levels were observed (89). Lastly, the ethanolic leaf extract of *Moringa oleifera* rescued the immune cells from cytotoxicity induced by melamine, a widespread food contaminant and adulterant.

CONCLUSION

In vitro and *in vivo* evaluation of immunomodulatory effects of *M. oleifera* plant provides insightful information about the use of the plant in managing inflammatory and chronic diseases, Figure 3. However, more studies are needed to fully understand the effects of different *M. oleifera* plant preparation and isolated compound in human immune cells. The immunomodulatory effects of *M. oleifera* on mesenchymal stem cells are a potential area of research as little data is currently available. Overall, different extract preparation of *M. oleifera* plant extract can modulate the immune response of immune-modulatory cells in a context-dependent manner, resulting in a favourable immune response, which might increase the general health and well-being of organisms.

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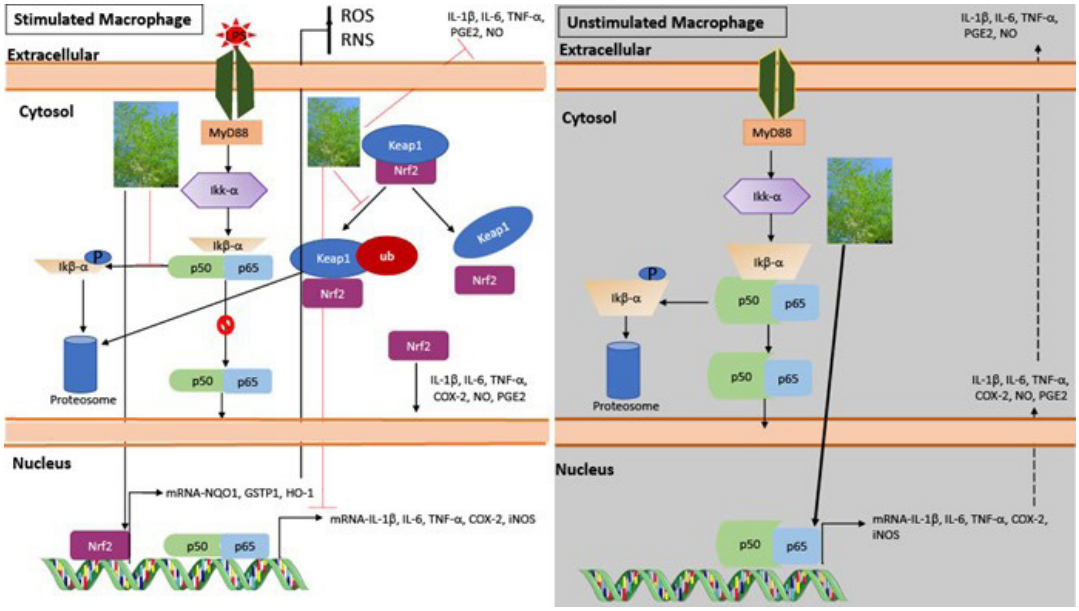

Figure 3: PImmunomodulatory effects of *Moringa oleifera* plant on Immune cell response

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