REVIEW

The Use of Neem/ Semambu (*Azadirachta indica)* Leaves in Coronavirus Disease (COVID-19)

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Disclosure

The authors of this review have no competing interest in this subject.

Disclaimer

This review is essentially a brief report, prepared on an urgent basis, to reflect the highest level of evidence available regarding the subject at this specific time. The conclusion draws on restricted reviews from analysis of pertinent literature, on expert opinion and/or regulatory status where appropriate. All efforts have been made to ensure all relevant published material has been reviewed but this document may still not fully reflect all scientific research available. Additionally, other relevant scientific findings may have been reported since completion of this review.

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Executive Summary

Title

The Use of Neem/ Semambu (*Azadirachta indica*) Leaves in Coronavirus Disease (COVID-19)

Objective

The objective of this report is to assess current available evidence on the potential of neem (*Azadirachta indica*) in COVID-19 management based on the following:

- Efficacy: Focus on neem reported properties of 1: antiviral, 2: modulation of immune response, and 3: role as other supportive therapy or management of disease related complications; and their respective potential mechanism(s) of actions.
- Safety of neem

Methodology

Electronic databases were searched using pre-determined terminologies such as ‘azadirachta indica’, ‘antiviral’, immunomodulatory’, ‘immune response’, ‘mechanism of action’, and ‘safety’. All clinical and preclinical studies (both in vitro and in vivo) related to safety and efficacy or effectiveness of *A. indica* in treating viral diseases were included.

Results & Discussion

Based on literature search, *Azadirachta indica* leaves were traditionally used for fever, measles and malaria, both orally and topically, but the amount ingested is not clearly documented. For antiviral effects, there is only one study conducted in humans, small study with 10 patients, while the others were mainly in vitro and in silico simulation docking studies that cover hepatitis C, dengue and polio viruses. As there is no animal study, the possible range of effective doses is not established. From the stimulating aspect of the immune system cells, most of the studies were conducted in vitro on bacteria and not viruses. As for safety, in adults, studies on the plant suggest that oral ingestion might be safe if it is consumed for a short period of time and in small doses but the dose is not well established. Toxicity studies in animal studies have shown high variability with side effects occurring at low doses. Furthermore, ingestion is not safe in children as it can cause fatal neurological reactions; and among pregnant women, which can terminate pregnancy. There is insufficient data to suggest that neem is safe among breastfeeding mothers, hence consumption should be avoided. Animal and in vitro studies also suggested

precaution is needed in conditions like diabetes, autoimmune disorders and transplants.

Conclusion

Most activities of neem (*Azadirachta indica*) are only reported in preclinical study models with insufficient high quality of clinical evidence. Although many phytochemical compounds have been identified from neem, these have not been sufficiently investigated in preclinical and clinical trials to solidly support use as antiviral, especially in COVID-19. The reported adverse reactions, especially in large doses for adults and even in small doses among young children should be considered and weighed against its unclear potential benefits from current available data.

Full report on neem potential in COVID-19 management

Plant information

- Scientific name: *Azadirachta indica*

- Common name (1):
    - English: Bead tree, cornucopia, Indian lilac, limbodi oil, margosa, margosa tree, neem, neem oil, neem tree, nim
    - Malay: Baypay, mambu, repe, veppam, weppa

- Chemical Constituents (2):
    - Important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol.
    - Quercetin and **β**-sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties and seeds hold valuable constituents including gedunin and azadirachtin

Traditional Use

- In India, *A. indica* leaves are commonly cooked as food and prescribed for fever (3).
- For fever; three twigs of *A. indica* are crushed and mix well in a pail of water. The preparation is used as bathing water 3 times a day (morning, evening & night) till cured. (4)
- For general wellness; 12–13 *A. indica* leaves are boiled in water, cooled then consumed orally (4).
- For measles; A few leaves of *A. indica* are pounded and the extract is mixed with rice flour paste and applied on affected areas twice a day after bathing (4).
- *A. indica* is one of the most popular malaria remedies throughout Africa. In order to treat the symptoms of malaria, either a decoction of the bark or the leaves, and in some cases both, is ingested (5).
- In Indochina, tinctures of the *A. indica* leaves and bark are taken internally for malaria and as tonic (3).
- *A. indica* has also been used to treat malaria in the form of a vapor bath, often with numerous other herbs added into the bath (6).

Results & Discussion

Efficacy

Antiviral properties:

- A human trial using acetone-water neem leaf extract was evaluated in 10 patients with HIV/AIDS at 1,000 mg daily for 30 days showed improvement in haemoglobin concentration, mean CD4+ cell count and erythrocyte sedimentation rate with no adverse effects were observed (7).

- Based on computational approach, four flavonoids from *A. indica* (kaempferol-3-*O*-rutinoside, rutin, hyperoside and epicatechin) showed potential as inhibitors to dengue virus (DENV) serine protease. Subsequently, kaempferol-3-O-rutinoside and epicatechin were selected for cell viability (MTT) assay and in vitro antiviral (focus forming unit) assay. Results suggested that kaempferol-3-*O*-rutinoside and epicatechin exhibit strong and moderate inhibition activity against DENV-2 strain respectively as compared to reference compound quercetin ($p < 0.0001$) with no significant cell toxicity. (8)

- Phytochemicals of *A. indica* leaves have antiviral activity against hepatitis C virus (HCV) NS3 protease through molecular docking and simulation

approach. Results show that the compound 3-deacetyl-3-cinnamoyl-azadirachtin possesses good binding properties with HCV NS3/4A protease. It can be concluded from this study that deacetyl-3-cinnamoyl-azadirachtin may serve as a potential inhibitor against NS3/4A protease with phytochemicals such as 3-deacetyl-3-cinnamoyl-azadirachtin with HCV NS3/4A, alpha-linolenic acid with NS3/4A, azadirachtanin with DENV NS3/4A and 23-*O*-methylnimocinolide with NS3/4A. (9)

- Two polysaccharides from the leaves of *A. indica* show significant in vitro antiviral activity towards poliovirus. Better inhibitory effect observed when the polysaccharides from the leaves added concomitantly with the virus infection with a dose dependent curve inhibition.It is concluded that *A. indica* acts against PV-1 by inhibiting the initial stage of viral replication. (10)

Immunomodulatory properties:

- The immunomodulatory effect of neem (*A. indica*) seed's aqueous, ethanolic extracts and *Candida albicans* cell wall mannoproteins on the immune response of mice vaccinated with Brucella Rev-1 vaccine was studied. Aqueous and ethanolic neem seed's extract exhibited the highest augmentation in all immunological parameters engaged in comparison with mannoproteins of *C. albicans* cell wall. (11)

- The number of ingested cells was found to be 60 out of 100 after 1.5 hours incubation of blood samples with *Staphylococcus aureus* and *A. indica.* The result for *A. indica* showed enhanced phagocytic activity as compared to control blood samples without the herb. This suggests that diet or drug supplemented with *A. indica* (neem) is likely to augment the immune system and reduce the growth of pathogenic bacteria like *S. aureus.*(12)

- Weekly intraperitoneal administration of neem leaf glucosamine to male Swiss Wistar mice (10 mice/group) at three different doses (266 µg/ 30 g mouse, 400 µg/ 30 g mouse & 800 µg/ 30 g mouse) for four weeks demonstrated significant elevation of Interleukin-2 (IL-2) concentration in mice serum as compared to untreated control group ($p < 0.05$). Proliferation of T-lymphocytes in thymus was also observed in all treated groups based on the histopathology examination of the thymus. These findings postulated the potential of neem leaf glucosamine as an immunostimulant. (13)

Safety

Adults: (Human studies):

- Oral: **POSSIBLY SAFE**, for short term: 60 mg up to 10 weeks for bark extract (14); **POSSIBLY UNSAFE** in large doses and long term. (150mL for 5 days (15); 250 mL of NeemAzal-T/S, 1% Aza-dirachtin (16); 20mL (17) and Neem tea, several litres/day for 3 weeks (18)). As reported in case reports using neem oil (plant part not specified), may harm kidney and liver; may cause acidosis and encephalopathy.

Children: (Human studies):

Oral: **LIKELY UNSAFE**, case reports of serious side effects in infants and small children can happen within hours after taking neem oil such as vomiting, diarrhea, drowsiness, blood disorders, seizures, loss of consciousness, coma, brain disorders, and death were found. (Dose varies at 5mL (19-20); dose not specified (21); few drops and 5mL (22))

Breastfeeding & Pregnancy:

LIKELY UNSAFE. Animal studies show that neem seed extract can cause miscarriage and terminate pregnancy in rodents and primates. Dose varies at 0.6mLx 3 days, D8-D10 pregnancy in rats- no concentration (23); 3-6mL/day x 6 days in monkeys and baboons- no concentration for both study (24)

Precautions in diseases:

Diabetes: Animal studies showed it may lower blood sugar (25).

Autoimmune diseases: Animal studies show immunomodulatory effect, may affect disease progression and interact with drugs (26).

Transplant patients: Animal studies show immunomodulatory effect, may affect disease progression and interact with drugs (26).

Anti-Fertility: In vitro study showed spermicidal activity (leaf) (27), male antifertility effects in male mice (28), anti-spermatogenesis in rats (neem seed oil (29), neem leaf (30)), disrupt ovulation in rats (neem flower extract (31)). Human study shows potential anti HCG (neemseed oil) effects when given in combination with heterospecies dimer birth control vaccine (32).

Allergy reactions: Allergic contact dermatitis has been reported in human case

report (33).

Preclinical toxicity study

- The leaf and seed water extract of *A. indica* produced acute toxicity effects when intramuscularly administered in male rats (lethal dose, 50%, LD_{50} for leaves = 0.57–0.62 g/kg) (34).

- The water and methanol extracts of *A. Indica* leaves were studied in male Wister rats following treatment with 20% w/w and 30% w/w equivalent of water and methanol extracts of neem leaves incorporated into rat diet and administered orally for 90 days did not show any changes on the hematological parameters. (35)

- Methanolic extracts of neem leaf and bark had oral LD_{50} values of about 13 mg/kg in mice, and the poisoning signs were discomfort, gastrointestinal spasms, loss of appetite, hypothermia, and, ultimately, convulsion leading to death within 24 hours (36).

- The administration of neem extract containing 12% azadirachtin for 90 days in rats did not find any histopathological, hematological, enzymatic or other adverse effects at 500, 1,000 and 1,500 mg/kg daily oral doses; a single oral dose of 5,000 mg/kg of this substance produced neither toxic symptoms nor death (37).

- Oral, intraperitoneal and intramuscular administration of an aqueous suspension of dried neem leaves in mice caused no deaths at a dose of 800 mg/kg body weight but was lethal at 1,000 mg/kg body weight. (38)

- Administration of ethanolic extract of neem leaves intravenously to anaesthetised rats at doses of 100, 300 and 1,000 mg/kg caused bradycardia (at 100 mg/kg) and ventricular arrhythmias (at 300 mg/kg). Also produces dose-dependant reduction in blood pressure from 100, 300, and 1,000 mg/kg. (39)

- The body weight of goats and guinea pigs decreased due to administration of leaves to their drinking water. Both acute and chronic toxicity were evident through signs of weakness, loss of condition and depression. Decreases in heart, pulse and respiratory rates were observed and diarrhoea, tremors and ataxia occurred in some animals. Total erythrocyte count (TEC), packed cell

volume (PCV) and haemoglobin (Hb) decreased slightly, whereas the activities of SGOT, sorbitol dehydrogenase and the concentrations of cholesterol, urea, creatine and potassium increased. Liver and kidneys were most affected. (40)

- Rats treated with leaf extract of neem at 100 mg/kg body weight showed decreased appetite, body weight and pupillary reflex. Their TEC and blood glucose level were reduced. Histopathological studies revealed congestion in the liver, kidneys, lungs and brain. (41)
- Crude neem leaf extract causes genotoxicity in male mice germ cells at a dose of 0.5–2 g/kg body weight for six weeks. Some structural change in meiotic chromosomes along with chromosome strand breakage or spindle disturbances and abnormal regulation of genes controlling sperm shape were observed. (42)

- Single dose intraperitoneal injection of Neem leaf glucosamine in male Swiss Wistar mice (six mice/group) did not cause any acute toxicity effect at doses of 1 mg, 2 mg & 4 mg/ 30 g mouse (13).

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Conclusion

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