Mini Review

Importance of Neem Leaf: An insight into its role in combating diseases

Dharmendra Kumar Yadav1, Yogesh P. Bharitkar2, Kasturi Chatterjee1, Monisankar Ghosh3,
Nirup Bikash Mondal2 & Snehasikta Swarnakar1*

1Cancer Biology & Inflammatory Disorder Division; 2Organic & Medicinal Chemistry Division; and
3Drug Development Diagnostic and Biotechnology Division, CSIR-Indian Institute of Chemical Biology (IICB),
Kolkata-700 032, West Bengal, India.

Received 30 March 2016; revised 04 May 2016

The neem (Azadirachta indica A. Juss) is a tropical evergreen tree (Fam. Meliaceae; Subfam. Melioideae) traditionally well known for its medicinal value. Beneficial effects of different parts of neem are attributed to its biologically active principle ‘Azadirachtin’. Apart from Indian subcontinent, neem is widely used in African countries as therapeutics, preservatives and insecticides. Neem leaves, natural source of flavonoids, polyphenols, isoprenoids, sulphurous and polysaccharides, play important role in scavenging the free radical and subsequently arresting disease pathogenesis. Considerable research has gone into neem for developing cost effective and non-toxic products. The present review has compiled different phytochemicals isolated from neem leaves, methods of extraction and their therapeutic use in preventing several diseases. Here, we highlighted the mechanism of anti-inflammatory and antioxidant activity of neem leaf that underscores the disease through regulation of physiological responses. Also, multiple roles of neem leaf and commercial use of neem formulation as an alternative in paving a frontier in the field of drug discovery are discussed.

Keywords: Angiogenesis, Apoptosis, Antidiabetic, Antifungal, Antimicrobial, Antioxidant, Anti-inflammatory, Antiulcer, Antiviral, Ayurvedic, Azadirachta indica, Hepatoprotective, NF-κB pathway, ROS, Traditional medicine, Unani, Wound healing

Introduction

Azadirachta indica A. Juss., commonly called ‘Neem’, has been used in traditional medicine since antiquity, is regarded as ‘holy tree’1,2. Neem trees grow generally 15-20 m tall and have a life span of 150-300 years. Fruits are green that turns yellow on the time of ripening. It is sensitive to frost and restricted to warmer parts of the world (21-32ºC). Though known to occur in at least 78 countries worldwide, including Asia, Africa, Australia, north, central and South America and partly in Europe, it is native to India, Indonesia, Malaysia, Myanmar, Pakistan, Senegal, Sri Lanka, Bangladesh, Nepal and Thailand1. The exotic countries where the neem tree has been found are Algeria, Angola, Antigua, Barbuda and Sudan3. It grows in varieties of soil such as clay, sandy, saline and alkaline with a wide pH 4-104,5.

India, with 20-25 million trees, account for 60% of the total worldwide strength of neem trees. Uttar Pradesh represents the highest (56%) followed by a Tamil Nadu (15%) and Karnataka (6%) at second and third place, respectively5,6 (Fig. 1).

While different parts of neem tree e.g. leaf, seed, bark, flower, fruit and root show various biological activities and therapeutic potential6, in this review, we

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*Correspondence:
Phone: +91 33 24995824; Fax: +91 33 24735197
E-mail: snehasiktas@hotmail.com

Abbreviations: ANLE, aqueous neem leaf extract; DMBA, dimethylbenz(a)anthracene; ENLE, ethanolic neem leaf extract; HBP, hamster buccal pouch; IL, interleukin; MNLE, methanolic neem leaf extract; MNNG, methyl nitro-nitrosoguanidine; NLEs, neem leaf extracts; NF-xB, nuclear factor kappa B; ROS, reactive oxygen species; TNF, tumor necrosis factor.

Fig. 1.—Occurrence of neem trees in different states of India. [The largest percentage of neem tree is found in Uttar Pradesh while Tamil Nadu and Karnataka occupy second and third place, respectively5,6].
focus up on its leaves. Of >200 compounds isolated from different parts of the neem, leaves share >50. Fresh young neem leaf contain 60% water, 23% carbohydrate, 7% protein, more than 7% minerals, 0.89-3.96% calcium, 0.11-0.30% phosphorus and total ash of 7.73-18.37%. Neem leaf is rich in fatty acids and amino acids, mainly, glutamic acid, tyrosine, alanine, glutamine and cysteine. Neem leaf extracts (NLEs) and its constituents exhibit antibacterial, anticarcinogenic, antihyperglycemic, anti-inflammatory, anti-mutagenic, anti-inflammatory, antinociceptive, antioxidant, cardioprotective, hepatoprotective, and immuno-modulatory properties. Further, tribes in India used neem leaves to treat ecto- and endo-fatty acids and amino acids, mainly glutamic acid, from Neem (Fig. 2). Few important ones are Azadirachtin (C35H44O16), Ascorbic acid (C6H8O6), Chlorogenic acid (C16H18O7), Hyperoside (C21H20O12), Kaempferol (C15H10O6), Kaempferol-3-o-rutinoside (C27H26O15), Myricetin (C15H10O6), Nimbafalavone (C26H26O3), Nimbandiol(C25H32O3), Nimbin (C30H36O5), Nimbin,6-deacetyl (C20H36O3), Nimbine (C26H30O2), Nimbocinolide (C32H42O10), Nimbocinone (C29H40O4), Nimboide (C27H23O7), Nimocin (C15H32O2), Nimocinol (C28H36O3), Nimocinoline (C28H36O7), Quercetin (C15H10O7), Quercetin-rhamnoside (C26H22O11), Rutin (C27H30O16), Scopoletin (C10H9O4), Stigmastanol (C28H44O), Valasinin (C26H36O5) and Zafaral (C26H46O6) as shown in Fig. 2.

Earlier Indian chemists isolated a bitter pain reliever from neem oil and later on crystalized compound named nimbin from neem leaf; however, an easy method for separation of these bitter compounds is available. Dried plant material is generally used for compound extraction using percolation/ maceration and soxlet extraction along with supercritical fluid extraction, microwave-assisted extraction as well as counter current extraction. Different types of extract like petroleum ether extract (mixture of low polar compound), chloroform extract (mixture of intermediate polar compound), methanol extract (mixture of polar compounds) and methanol: water extracts (mixture of highly polar compounds) are used as mentioned in Scheme-I. Different extracts are treated differently for purification of phytochemicals. Low petroleum ether extract and chloroform extract generally are purified by normal phase column chromatography. Whereas, methanol extract, prior to chromatography, is put to n-Butanol:water fractionation for removal of inorganic impurities. The rotary dried n-Butanol part is then used for normal phase column chromatography with increasing order of polarity of mobile phase. Diaion HP-20 and Sephadex LH20 are used as adsorbent in column chromatography for purification of methanol: water extract. The spot identification of compounds is done using silica TLC or RP-18 TLC plates. Moreover, physical constants, spectral data Infrared Spectroscopy (IR), Electrospray Ionisation Mass Spectroscopy (ESI-MS), Proton Nuclear Magnetic Resonance (H NMR), Carbon-13 Nuclear Magnetic Resonance (13C NMR) with Distortionless Enhancement by Polarization Transfer (DEPT) 90 and 135, Two-Dimensional NMR–Correlation Spectroscopy (2D NMR–COSY), Nuclear Overhauser effect spectroscopy (NOESY), Heteronuclear Single Quantum Coherence (HSQC), Heteronuclear Multiple Bond Correlation (HMBC) and single X-ray crystallography are used for structural elucidation of the isolated compound.

### Table 1 — Antioxidant and anti-inflammatory activities of various compounds from neem leaf

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bio-activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azadirachtin</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Kaempferol/derivatives</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Myricetin</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Nimbin</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Nimbolide</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Rutin</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Scopoletin</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Sigmasterol/β-Sitosterol</td>
<td>Antioxidant</td>
</tr>
</tbody>
</table>

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Fig. 2—The major chemical compounds of neem leaf. [The name and structure of various isoprenoid, non-isoprenoid and miscellaneous compounds were described\textsuperscript{31,43,44}].

Scheme I—Outline of compound extraction and isolation procedure from neem leaf. [Dried plant material was used for extraction by several solvents. Different extracts were further processed to get purified compound and to determine their structures]
Neem Leaf Extracts (NLEs) in treating diseases

Several studies have reported medicinal potential of different NLEs as well as isolated compounds against different diseases, including cancer\(^9\)\(^-\)\(^11\), ulcer\(^8\)\(^-\)\(^31\), diabetes\(^12\)\(^-\)\(^13\), etc. (Fig. 3). However, different phytochemicals in neem leaf cure diseases by targeting key signalling pathways involved in the pathogenesis (Fig. 4).

Anticancerous activity

Considerable reports are there showing the anticancer, antitumor activities of different NLEs and/or purified compounds in different animal and in vitro studies\(^9\)\(^-\)\(^11\)\(^,\)\(^37\). Alcoholic NLEs contains compounds like nimbin, azadirachtin, nimbatikam, nimbinit, nimbindiol and quercetin which are well known bioflavonoids and reported to show anticancer activity\(^10\). Quercetin isolated from methanolic neem leaf extract (MNLE) exhibited apoptotic activity on leukemia cancer cell such as K-562, Jurkat and U-937\(^14\). Nimolinone, 2,3-dehydrosalannol, 6-deacetyl nimbinene and other natural bioactive compounds isolated from ethanolic extract showed anti-inflammatory, antiangiogenic and antitumor activity. In addition, ethanolic neem leaf extract (ENLE) show beneficial effects in the prevention of prostate cancer\(^48\).

Effect of neem on apoptosis and angiogenesis

Nimbolide, an active neem component from neem leaf significantly inhibits cell viability by inducing apoptosis, and suppressed cellular invasion and migration through abrogating STAT3 activation. It suppressed tumor growth and metastasis in transgenic adenocarcinoma of mouse prostate cancer model\(^49\). Neem limonoids viz. azadirectin and nimbolide have been reported to induce cell cycle arrest and mitochondria-mediated apoptosis in cervical cancer (HeLa) cell line\(^27\). Ethanolic neem leaf extract (ENLE) induces apoptosis in PC-3 and LNCaP prostate cancer cells by PI3K/Akt mediated pathway\(^50\). It exerts chemopreventive effect by modulating lipid peroxidation and enhancing the antioxidant status in the stomach\(^11\). ENLE at the doses of 250-500 mg/kg body wt. showed protective role against fore-stomach papillomagenesis; and downregulated c-Myc expression in induced breast cancer model\(^51\). In hamster buccal pouch (HBP) carcinogenesis model, administration of NLEs significantly modulated the key hallmark events of cancer cells such as cell proliferation, angiogenesis and apoptosis due to its antioxidant properties\(^52\).

Effect of neem on NF-κB pathway

Ethyl acetate chloroform insoluble fraction and the methanol ethyl acetate insoluble fraction of neem leaf inhibited tumorigenesis through modulation of xenobiotic-metabolizing enzymes and induced mitochondrial apoptosis through NF-κB signaling pathway in HBP carcinomas model\(^53\). Studies further documented that ENLE effectively suppressed dimethylbenz(a)anthracene DMBA-induced HBP carcinogenesis as revealed by the absence of tumours in the initiation phase and reduced tumour recurrence in post-initiation phase. Additionally, it suppressed lipid peroxidation and enhanced antioxidant status in the pouch, liver and erythrocytes as well as decreased formation of bone marrow micronuclei. ENLE exerted protective effects against methylnitronitrosoguanidine (MNNG)-induced genotoxicity by augmenting host.
antioxidant defence mechanisms\textsuperscript{54}. The effect of NLEs has been delineated in rat colon carcinogenesis model where it exerted chemopreventive role on endpoint preneoplastic lesions to ultimately arrest the induction of aberrant crypt foci\textsuperscript{55}. Furthermore, NLEs arrested DMBA-induced skin tumor in mice by increasing glutathione contents and antioxidant enzymes\textsuperscript{56}.

**Hepatoprotective effect**

Aqueous neem leaf extract (ANLE) showed hepatoprotection against anti-tubercular drug induced damage in rat as indicated by minimized alteration of bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in serum\textsuperscript{57}. Koul et al\textsuperscript{33} have demonstrated its ameliorative nature in DMBA-induced hepatotoxicity. Azadirachtin-A, one of the major components of neem, showed hepatoprotective effect against carbon tetrachloride (CCl\textsubscript{4})-induced liver injury in rat model. Pretreatment with azadirachtin-A inhibited cellular necrosis of hepatocytes as revealed by the histological and ultra structural studies\textsuperscript{58}. Another important constituent of neem, nimbolide also possess hepatoprotective effect against CCl\textsubscript{4}-induced liver damage with efficacy similar to that of silymarin standard\textsuperscript{59}.

**Wound healing and antiulcer effect**

Traditionally in India, neem leaf has been used for thousands of years for its ulcer healing properties\textsuperscript{60}. Current researchers have also established healing power of NLEs in different wound models\textsuperscript{6,31} including significant wound healing in Sprague Dawley rats for excision and incision wounds\textsuperscript{60}; increased neo-vascularisation promoting wound healing\textsuperscript{61}; and protection against ethanol-induced gastric mucosal damage\textsuperscript{62}.

**Antidiabetic activity**

Neem compounds such as rutin and quercetin have been shown to have hypoglycaemic/antihyperglycemic effects recently\textsuperscript{11,12}. NLEs showed actions in alloxan-induced diabetic rats by significantly reducing glucose levels as compared to control group\textsuperscript{63}. Chloroform extract of \textit{A. indica} leaves showed promising results for oral glucose tolerance test and significantly reduced the intestinal glucosidase activity in murine model of diabetes\textsuperscript{64}. Meliacinolin, a new tetrannortriterpenoid from chloroform extracts of \textit{A. indica} leaf, also demonstrated \textit{in vivo} antidiabetic property against streptozotocin-induced type 2 diabetes in mice\textsuperscript{65}. Meliacinolin treatment restored blood glucose level, serum biochemical parameters, hepatic enzymes, thiobarbituric acid reactive substances as well as insulin levels in streptozotocin-induced diabetic mice to normal levels. It also inhibited α-glucosidase and α-amylase activities which could be an effective strategy to lower the levels of postprandial hyperglycemia\textsuperscript{65}.

**Antimicrobial effect**

For more than thousand years, neem leaf is traditionally used in India for its antimicrobial (antibacterial, antifungal and antiviral) properties\textsuperscript{59}. \textit{In vitro} study against \textit{Enterococcus faecalis} has shown potential antimicrobial roles of NLEs\textsuperscript{66}. In another report, antibacterial properties of NLEs were studied against 21 strains of food borne pathogens and neem showed promising results\textsuperscript{67}. NLEs also showed potent antimicrobial effects against bacteria isolated from adult mouth\textsuperscript{68}. Additionally, the extracts were also effective against bacteria like \textit{Streptococcus mutans}, \textit{S. salivarius} and \textit{Fusobacterium nucleatum}, etc\textsuperscript{69}. Methanolic and ethanolic extract of neem leaf showed antimicrobial activities against human pathogenic bacteria such as \textit{Pseudomonas areuginosa}, \textit{Bacillus pumillis}, \textit{Staphylococcus aureus}\textsuperscript{8,70}. Different extracts of neem leaf exhibited antimicrobial effect against \textit{Proteus mirabilis} and \textit{Bacillus cereus} which infect reproductive tract in women\textsuperscript{71}. Vimala et al\textsuperscript{72} has shown the use of silver nanoparticle conjugated neem compound for advanced antibacterial applications. Neem cake is also reported to inhibit bacterial load and maintain the quality of preserved meat\textsuperscript{73}.

**Antiviral activity**

Antiviral properties of NLEs are still not well investigated. Two polysaccharides isolated from the leaves of \textit{A. indica} have demonstrated better virucidal effect than their sulfated derivatives \textit{in vitro}. They inhibited poliovirus type 1 (PV-1) at the initial stage of viral replication\textsuperscript{74}. In another study, NLEs showed virucidal activity against coxsackie virus B-4 through virus inactivation and yield reduction\textsuperscript{75}.

**Antifungal effect**

Local communities from southern Odisha, India use neem leaf paste as ethnoveterinary application for skin infections including fungal, in small ruminants\textsuperscript{39}. Studies have claimed that alcoholic and aqueous extracts of neem leaf inhibited the growth of seed borne fungi like \textit{Aspergillus} and \textit{Rhizopus}\textsuperscript{76}. The
aqueous, ethanolic and ethyl acetate extracts of neem leaves inhibited growth of some human pathogens such as *Aspergillus flavus*, *A. fumigates*, *A. niger*, *A. terreus*, *Candida albicans* and *Microsporum gypseum*. Also, NLEs have been reported to inhibit growth of fungi like *Alternaria solani*, and *Cladosporium*.

**Other effects**

Among other effects, NLEs exhibit antimalarial effect on *Plasmodium berghei* infected albino mice. MNLE prevents cisplatin-induced nephrotoxicity by limiting oxidative stress and apoptotic responses in rat model. Neem leaf-based mouth rinse has also reported to show effective protection against dental inflammations, like gingivitis. It also reduced hypoperfusion-induced gliosis, perivascular lymphocytic infiltration, recruitment of macrophages and cellular edema following long term hypoperfusion. NLEs also exhibited spermicidal properties in different in vivo (upon intravaginal application) and in vitro experiments. Azadirachtin is reported to cause rapid loss of mobility and reduced fitness in insects. It supports the claim that neem based compounds are better natural pesticide and insecticide.

Although various neem components have shown every indication of being safe to mammals in normal use, the possibility of future hazards should not be ignored. Its residual persistence on foods is also unknown.

**Mechanisms of Antioxidant and Anti-inflammatory activities of Neem Leaf**

Exposure to oxidant molecules robustly leads to the generation of reactive oxygen species (ROS for example, H$_2$O$_2$, O$_2^-$, -OH) that can readily alter DNA, proteins and/or membrane phospholipids. This phenomenology of oxidative stress is related to the pathophysiology of various diseases and considered responsible for several chronic disorders including cancer, diabetes, neurodegenerative and cardiovascular diseases. Depletion of intracellular antioxidants in acute oxidative stress is intricately associated with ROS accumulation and therefore, it is essential to balance the ratio of antioxidants to oxidants by exogenous supplementation or by induction of cellular antioxidants enzyme. Terpenoids, alkaloids, flavonoids and glycosides are the major classes of constituents found within the neem leaf extract that in isolation or in combination simultaneously work as antioxidants, radical scavengers and anti-inflammatory compounds and therefore, might be exploited for diverse therapeutics application. Table 1 represents the antioxidant and anti-inflammatory activities of various pure compounds isolated from NLEs and Fig. 5 depicts the antioxidant and anti-inflammatory mechanism of NLEs.

The anti-inflammatory and antioxidant property of NLEs have been documented in different in vitro and in vivo studies. Free radical generation causes DNA strand breakage and NLEs reverse this phenomenon by scavenging the radical to their non-radical form. The action of neem leaf against oxidative damage was reported even in cancer model, where treatment of azadirachtin and nimboide exhibited concentration-dependent anti-radical scavenging activity. The antioxidant capacity of NLEs were evidenced through reduced H$_2$O$_2$-mediated lipid peroxidation and DNA damage. Evidences indicate that NLEs efficiently arrest oxidative stress in vivo, without a manifestation of toxicity and therefore, might be considered an excellent antioxidant agent for therapeutic use. Moreover, treatment with NLEs cause a substantial increase in liver function by increasing glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase activities. NLEs showed an
anti-inflammatory potential where it inhibited TNF-α triggered induction of NF-kB that is exclusively linked to inflammation and ultimately aggressive cancer outcomes. Anti-inflammatory effects of chlorogenic acid, which is a major compound found in neem leaf, was observed in lipopolysaccharide-stimulated RAW 264.7 cells. Chlorogenic acid significantly inhibited not only NO production but also the expression of COX-2, nuclear translocation of NF-kB, iNOS along with other cytokines (IL-1β, IL6 and TNF-α) in a dose-dependent manner. Moreover, NLEs potentially inhibited histamine, bradykinin and PGE1 induced inflammatory edema in rat paw. Similar observations were found where oral administration of ANLE at a dose of 200 mg/kg exerted significant regression of granuloma formation in cotton pellet granuloma assay further suggesting the anti-inflammatory potential of neem leaf. Studies demonstrated that NLEs led to the stabilization of lysosomal enzymes and produced significant antinociceptive activity in rats.

Future Direction for Common Use of Neem

Natural products and their derivatives have been endowed with life-giving resources including therapeutics not only true for the past but also in modern time. With recent development in sciences and technologies, the identification of a single biologically active ‘lead compound’ is much easier now. HPLC-mass spectrometer (LC-MS) systems and natural-product databases are great help in identifying new compounds and avoiding replication of previous efforts. Moreover, novel de novo structure determination of compounds has been revolutionized through advancements in spectroscopic and high-resolution nuclear magnetic resonance technologies. Compounds with potential for drug development may be applied preliminary in structure–activity relationship (SAR) studies which fish out the active compounds to scale up the purification process. Furthermore, with synthetic modification if any compound fits the criteria of evaluation against specific disease, the compound is declared as a ‘lead’ and further use for experimental trial methods.

Commercial use of neem formulations should be developed and made available for common use instead of crude neem preparation. Crude formulations often degrade rapidly and therefore, formidable to know precise application. Relevant research is necessary to explore the benefit of neem product, so as to boost the neem products research and utilization. Commercial preparation of some botanical pesticides should include synergist such as piperonylbutoxide to increase the effectiveness of the pesticide. Moreover, studies on usage of neem leaf as fertilizer cum pesticide vermicompost has shown significant beneficial impact for eco-friendly agriculture.

In general, awareness on the economic significance of neem, especially its pesticidal potential amongst farmers is the need of the hour. Professional educational programs are needed for organic pesticide education and in related fields of biochemistry and natural products. An organizational infrastructure has to be in place to plan, foster, facilitate and coordinate research so that the current gap in scientific research findings on neem including biopesticide development and utilization could be consciously bridged. NeemAura Naturals is the first organization to develop certified organically neem leaf products such as toothpaste, neem cream, hand and body lotion, neem herbal spray and neem soap. Neem Co-op was formed in 1996 Northern New South Wales (NSW), Australia manufacturing and selling a broad range of neem products. India is making varieties of neem products such as agricultural products, soap, food and veterinary products and they are used in India as well as Asian and African countries. Further studies on neem leaf offer the potential for much effective therapeutics against multiple ailments in cost-effective ways.

Conclusion

In this review, we summarize various components of neem leaf and their bioactive potential for anticancer, anti-inflammatory, antibacterial, antiulcer, antioxidant, antidiabetic effects. Literature survey reveals that extraction of neem leaf in different solvents contain several phytochemicals such as flavonoids, polyphenols, etc., and these phytoconstituents possess potential preventive role for several diseases. On the basis of studies, it is confirmed that neem leaf extract or particular component show anti-inflammatory and antioxidant action during prevention of various diseases. Multifaceted therapeutic implication of neem leaf has been accepted traditionally all over the world, particularly in Indian subcontinent because of easy availability, less toxicity and affordability since more than 2000 years. Although, phytochemicals are increasingly being used as potential therapeutic agent
for different diseases, there remains a continuous gap in information how the new agents (drugs) target the particular physiological or cellular response. This review will be helpful for researchers in phytotherapy work using neem tree in general or specially neem leaf for new drug discovery.

Acknowledgement

The authors acknowledge the financial support from the Council of Scientific and Industrial Research (CSIR), New Delhi and BSC/0111 research fund. DKY is the recipient of CSIR-senior research fellowship. The authors also thank Dr. Satyabrata Ghosh of Jadavpur University, Kolkata and Dr. Soumenndranath Talapatra of Career Advancement Solution, Kolkata for helpful inputs and suggestions.

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