REVIEW ARTICLE

Biological activities and medicinal properties of neem (*Azadirachta indica*)

Kausik Biswas, Ishita Chattopadhyay, Ranajit K. Banerjee* and Uday Bandyopadhyay

Department of Physiology, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Kolkata 700 032, India

Neem (*Azadirachta indica* A. Juss) is perhaps the most useful traditional medicinal plant in India. Each part of the neem tree has some medicinal property and is thus commercially exploitable. During the last five decades, apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products. This review gives a bird's eye view mainly on the biological activities of some of the neem compounds isolated, pharmacological actions of the neem extracts, clinical studies and plausible medicinal applications of neem along with their safety evaluation.

MEDICINAL plants are part and parcel of human society to combat diseases, from the dawn of civilization. *Azadirachta indica* A. Juss (syn. *Melia azadirachta*) is well known in India and its neighbouring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. *A. indica* A. Juss and *M. azedarach* are two closely related species of Meliaceae. The former is popularly known as Indian neem (margosa tree) or Indian lilac, and the latter as the Persian lilac. Neem is an evergreen tree, cultivated in various parts of the Indian subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity[1–6]. Neem has been extensively used in ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine. The sanskrit name of the neem tree is 'Arishtha' meaning 'reliever of sickness' and hence is considered as 'Sarbaroganibarini'. The tree is still regarded as 'village dispensary' in India. The importance of the neem tree has been recognized by the US National Academy of Sciences, which published a report in 1992 entitled 'Neem – a tree for solving global problems'. The advancement of neem research has earlier been documented[7,8].

*For correspondence. (e-mail: ranajitb@yahoo.com)

The neem tree has been described as *A. indica* as early as 1830 by De Jussieu[9] and its taxonomic position is as follows:

Order	Rutales
Suborder	Rutinae
Family	Meliaceae (mahogany family)
Subfamily	Melioideae
Tribe	Melieae
Genus	*Azadirachta*
Species	*indica*

The genus *Azadirachta* A. Juss which comprises three species of Indo-Malayan origin has been characterized in detail[10,11].

Chemical investigation on the products of the neem tree was extensively undertaken in the middle of the twentieth century. Since the early report by Siddiqui[12] in 1942 on the isolation of nimbin, the first bitter compound isolated from neem oil, more than 135 compounds have been isolated from different parts of neem and several reviews have also been published on the chemistry and structural diversity of these compounds[5,6,13–19]. The compounds have been divided into two major classes: isoprenoids and others[18]. The isoprenoids include diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C-secomeliacins such as nimbin, salanin and azadirachtin. The nonisoprenoids include proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc. The details of the chemistry of various compounds falling under these groups have already been reviewed[17,18]. Only a few compounds whose bioactivity has been studied are presented here. As the pesticidal and antifeedant activities of azadirachtin and the related compounds have been reviewed earlier[17–19], they have been excluded from this review.

Biological activity of some neem compounds

Although a large number of compounds have been isolated from various parts of neem, a few of them have

been studied for biological activity as shown in Table 1. The structure of some of these bioactive compounds has been presented in Figure 1.

Nimbidin, a major crude bitter principle extracted from the oil of seed kernels of *A. indica* demonstrated several biological activities. From this crude principle some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated[12,20]. Nimbidin and sodium nimbidate possess significant dose-dependent anti-inflammatory activity against carrageenin-induced acute paw oedema in rats and formalin-induced arthritis[21,22]. Antipyretic activity has also been reported and confirmed in nimbidin[23]. Oral administration of nimbidin demonstrated significant hypoglycaemic effect in fasting rabbits[24]. A significant antiulcer effect was observed with nimbidin in preventing acetylsalicylic acid, indomethacin, stress or serotonin-induced gastric lesions as well as histamine or cysteamine-induced duodenal ulcers[25,26]. Nimbidin can also suppress basal as well as histamine and carbachol-stimulated gastric acid output and may act as an antihistamine by blocking H_2 receptors, thereby helping as an antiulcer agent[27]. The spermicidal activity of nimbidin and nimbin (**1**) was reported in rats and human as early as 1959 (refs 28, 29). Nimbidin also demonstrated antifungal activity by inhibiting the growth of *Tinea rubrum*[30]. *In vitro*, it can completely inhibit the growth of *Mycobacterium tuberculosis* and was also found to be bactericidal[30]. Diuretic activity was also reported for sodium nimbidinate in dogs[31]. Nimbolide (**2**) has been shown to exert antimalarial activity by inhibiting the growth of *Plasmodium falciparum*[32,33]. Nimbolide also shows antibacterial activity against *S. aureus* and *S. coagulase*[34].

Gedunin (**3**), isolated from neem seed oil has been reported to possess both antifungal[35] and antimalarial[33] activities. Azadirachtin (**4**), highly oxygenated C-seco-meliacins isolated from neem seed and having strong antifeedant activity[17,19,36], has been demonstrated to have antimalarial property as well. It is inhibitory to the development of malarial parasites[37]. Mahmoodin (**5**), a deoxygedunin isolated from seed oil, has been shown to possess moderate antibacterial action against some strains of human pathogenic bacteria[18]. Condensed tannins from the bark contain gallic acid, (+) gallocatechin, (–) epicatechin, (+) catechin and epigallocatechin, of which gallic acid (**6**), (–) epicatechin (**7**) and catechin (**8**) are primarily responsible for inhibiting the generation of chemiluminescence by activated human polymorphonuclear neutrophil (PMN)[38], indicating that these compounds inhibit oxidative burst of PMN during inflammation. Three tricyclic

Table 1. Some bioactive compounds from neem

Neem compound	Source	Biological activity	Reference
Nimbidin		Anti-inflammatory	21
		Antiarthritic	22
		Antipyretic	23
		Hypoglycaemic	24
		Antigastric ulcer	25, 26
		Spermicidal	29
		Antifungal	30
		Antibacterial	30
		Diuretic	31
Sodium nimbidate		Anti-inflammatory	21, 22
Nimbin (**1**)	Seed oil	Spermicidal	28
Nimbolide (**2**)	Seed oil	Antibacterial	32, 33
		Antimalarial	34
Gedunin (**3**)	Seed oil	Antifungal	35
		Antimalarial	33
Azadirachtin (**4**)	Seed	Antimalarial	37
Mahmoodin (**5**)	Seed oil	Antibacterial	18
Gallic acid (**6**), (–) epicatechin (**7**) and catechin (**8**)	Bark	Anti-inflammatory and immunomodulatory	38
Margolone (**9**), margolonone (**10**) and isomargolonone (**11**)	Bark	Antibacterial	39
Cyclic trisulphide (**12**) and cyclic tetrasulphide (**13**)	Leaf	Antifungal	40
Polysaccharides		Anti-inflammatory	41
Polysaccharides GIa (**14**), GIb	Bark	Antitumour	42
Polysaccharides GIIa (**15**), GIIIa (**16**)	Bark	Anti-inflammatory	43
NB-II peptidoglycan	Bark	Immunomodulatory	44, 45

Numbers in parentheses indicate structures shown in Figure 1.

diterpenoids, margolone (**9**), margolonone (**10**) and isomargolonone (**11**) isolated from neem stem bark are active against *Klebsiella*, *Staphylococcus* and *Serratia* species[39]. Sulphur-containing compounds such as cyclic trisulphide (**12**) and tetrasulphide (**13**) isolated from the steam distillate of fresh, matured neem leaves have antifungal activity against *Trichophyton mentagrophytes*[40]. Several polysaccharides from neem exhibit various biological effects. A polysaccharide extracted from bark inhibits carrageenin-induced inflammation in mouse[41]. Two water-soluble polysaccharides GIa (**14**) and GIb isolated from the bark of *Melia azadirachta*, demonstrated strong antitumour effect with complete regression of the tumours, when administered in mice at a daily dose of 50 mg/kg for four days from 24 h after subcutaneous inoculation of Sarcoma-180 cells[42]. Two more polysaccharides, GIIa (**15**) and GIIIa (**16**) isolated from *M. azadirachta* bark also showed significant anti-inflammatory effect on carrageenin-induced oedema in mice[43]. Two polymers isolated from an aqueous extract of neem bark possess anticomplement activity, amongst which the compound NB-II, a peptidoglycan of lower molecular weight was found to be more potent[44,45]. Some active ingredients (phytosterol fraction) isolated from the lipid part of neem fruits, exhibit antiulcer activity in stress-induced gastric lesions[46].

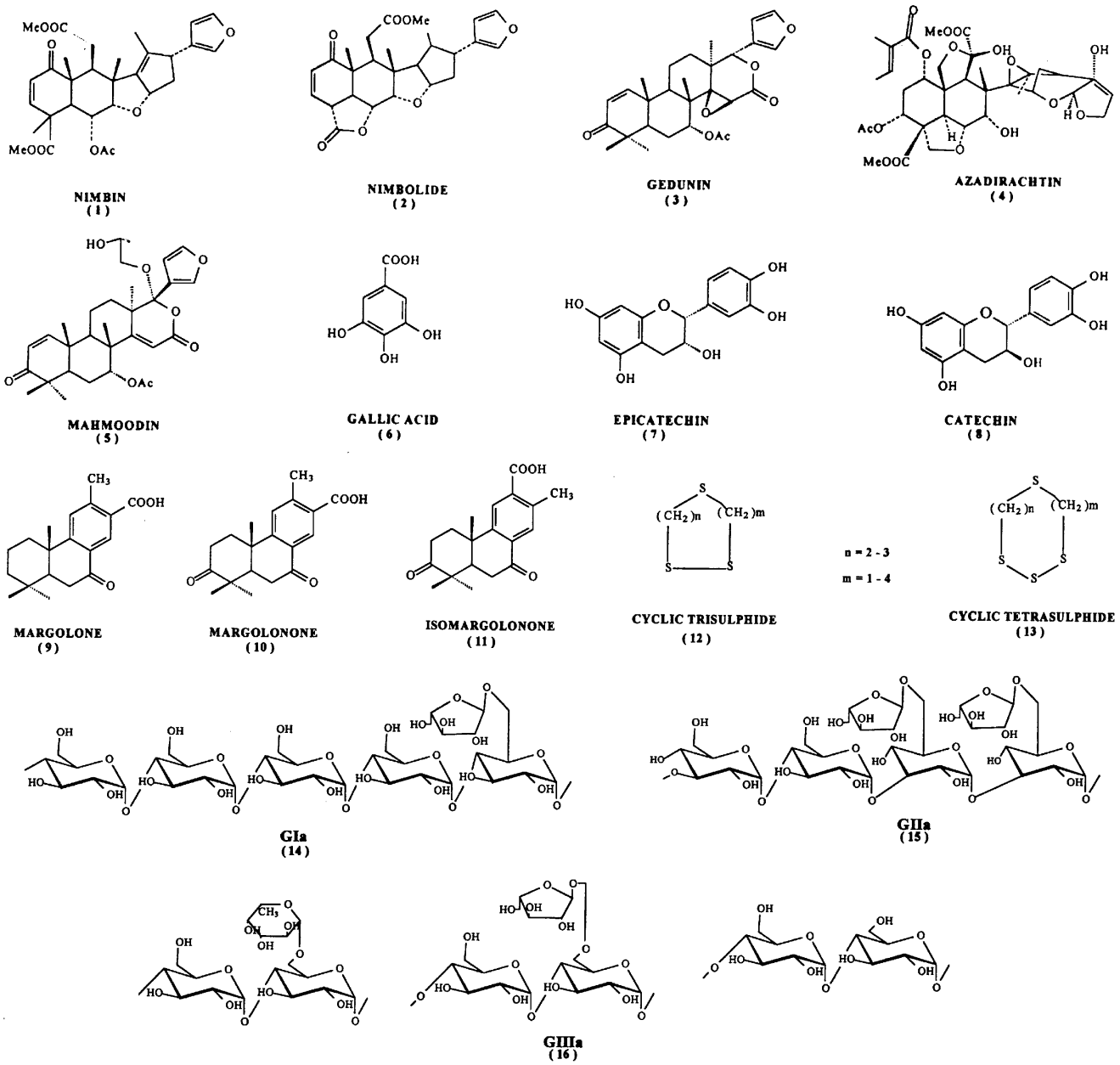

Figure 1. Structure of bioactive neem compounds.

Pharmacological actions of neem extract

Several pharmacological activities and medicinal applications of various parts of neem are well known[16,47]. Biological activity of neem is reported with the crude extracts and their different fractions from leaf, bark, root, seed and oil. However, crude extract of different parts of neem have been used as traditional medicine for the treatment of various diseases.

Medicinal use of various parts of neem

Various parts of the neem tree have been used as traditional ayurvedic medicine in India from time immemorial[48]. The medicinal utilities have been described, especially for leaf, fruit and bark[4]. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, constipation and also as a general health promoter[49]. Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident[3]. Neem oil finds use to control various skin infections[1]. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis[13]. Some of the medicinal attributes of various parts of neem[50] as mentioned in ayurveda[13,48] have been summarized in Table 2. However, apart from these uses, there are several reports on the biological activities and pharmacological actions of neem based on modern scientific investigations.

Anti-inflammatory, antipyretic and analgesic activities: The chloroform extract of stem bark is effective against carrageenin-induced paw oedema in rat and mouse ear inflammation[51]. Inflammatory stomatitis in children is cured by the bark extract[52]. Antipyretic activity has been reported in neem oil[30,53]. A methanol extract of the leaves exerts antipyretic effect in male rabbits[54]. The plant also possesses analgesic activity mediated through opioid receptors in laboratory animals[55]. Anti-inflammatory and antipyretic activities in various extracts have been reviewed[56].

Table 2. Some medicinal uses of neem as mentioned in ayurveda

Part	Medicinal use
Leaf	Leprosy, eye problem, epistaxis, intestinal worms, anorexia, biliousness, skin ulcers.
Bark	Analgesic, alternative and curative of fever.
Flower	Bile suppression, elimination of intestinal worms and phlegm.
Fruit	Relieves piles, intestinal worms, urinary disorder, epistaxis, phlegm, eye problem, diabetes, wounds and leprosy.
Twig	Relieves cough, asthma, piles, phantom tumour, intestinal worms, spermatorrhoea, obstinate urinary disorder, diabetes.
Gum	Effective against skin diseases like ringworms, scabies, wounds and ulcers.
Seed pulp	Leprosy and intestinal worms.
Oil	Leprosy and intestinal worms.
Root, bark, leaf, flower and fruit together	Blood morbidity, biliary afflictions, itching, skin ulcer, burning sensation and leprosy.

Immunostimulant activity: The aqueous extract of neem bark possesses anticomplement activity, acting both on the alternative as well as the classical pathway of complement activation in human serum[44]. Recently, an aqueous extract of stem bark has been shown to enhance the immune response of Balb-c mice to sheep red blood cells *in vivo*[57]. The aqueous extract of leaf also possesses potent immunostimulant activity as evidenced by both humoral and cell-mediated responses[58,59]. Leaf extract at 100 mg/kg after three weeks of oral administration causes higher IgM and IgG levels along with increased titer of antiovalbumin antibody[59]. Neem oil has been shown to possess immunostimulant activity by selectively activating the cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge[60].

Hypoglycaemic activity: Aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia[61]. The aqueous leaf extract when orally fed, also produces hypoglycaemia in normal rats and decreased blood glucose levels in experimentally-induced diabetes in rats[62]. Aqueous leaf extract also reduces hyperglycaemia in streptozotocin diabetes and the effect is possibly due to presence of a flavonoid, quercetin[63]. A significant hypoglycaemic effect was also observed by feeding neem oil to fasting rabbits[24]. Recently, hypoglycaemic effect was observed with leaf extract and seed oil, in normal as well as alloxan-induced diabetic rabbits[64]. The possible mechanisms underlying the hypoglycaemic activity of the aqueous leaf extract have also been discussed[65,66].

Antiulcer effect: Neem leaf aqueous extract produces antiulcer effect in rats exposed to restraint – cold stress or ethanol orally by preventing mucus depletion and mast cell degranulation[67]. An aqueous extract of neem bark has been shown from our laboratory to possess highly potent antiacid secretory and antiulcer activity and the bioactive compound has been attributed to a glycoside[68].

Antifertility effect: Neem oil proved spermicidal against rhesus monkey and human spermatozoa *in vitro*[69]. *In vivo* studies showed that intravaginal application of neem oil prior to coitus can prevent pregnancy[69]. Antifertility effect of neem oil has also been studied and suggested to be a novel method of contraception[70–72]. Oral administration of aqueous extract of neem leaf also shows

antifertility effect in mice[73]. Purified neem seed extract (Praneem) has also been demonstrated to abrogate pregnancy in both baboons and bonnet monkeys, when administered orally[74]. From the hexane extract of neem seed, an active fraction containing six components has been found to completely abrogate pregnancy in rodents when given orally up to a concentration of 10%, with no apparent side effect[75]. The effect is possibly due to activation of cell-mediated immune reaction. The mechanism of action of neem oil appears to be non-hormonal, probably mediated through its spermicidal effect and may have less side effects than steroidal contraceptives.

Antimalarial activity: Neem seed and leaf extracts are effective against malarial parasites[33,76]. Components of the alcoholic extracts of leaves and seeds are effective against both chloroquin-resistant and sensitive strains of malarial parasite[77]. Recently, neem seed extract and its purified fractions have been shown to inhibit growth and development of asexual and sexual stages of drug-sensitive and resistant strains of the human malarial parasite *P. falciparum*[78].

Antifungal activity: Extracts of neem leaf, neem oil and seed kernels are effective against certain human fungi, including *Trichophyton*, *Epidermophyton*, *Microsporum*, *Trichosporon*, *Geotricum* and *Candida*[79]. High antimycotic activity with extracts of different parts of neem has already been reported[56].

Antibacterial activity: Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *M. tuberculosis* and streptomycin-resistant strains[80]. *In vitro*, it inhibits *Vibrio cholerae*, *Klebsiella pneumoniae*, *M. tuberculosis* and *M. pyogenes*[81]. Antimicrobial effects of neem extract have been demonstrated against *Streptococcus mutans* and *S. faecalis*[82]. NIM-76, a new vaginal contraceptive from neem oil showed inhibitory effect on the growth of various pathogens, including bacteria, fungi and virus[83]. Recently, the antibacterial activity of neem seed oil was assessed *in vitro* against 14 strains of pathogenic bacteria[84].

Antiviral activity: Aqueous leaf extract offers antiviral activity against Vaccinia virus[85], Chikungemya and measles virus *in vitro*[86]. The antiviral and virucidal effects of the methanolic extract of neem leaves (NCL-11) have recently been demonstrated against group-B Coxsackie viruses[87]. NCL-11 inhibits plaque formation in different antigenic types of Coxsackie virus B at a concentration of 1 mg/ml at 96 h *in vitro*. Further studies indicated that NCL-11 is most effective in Coxsackie virus B-4 as a virusidal agent, in addition to its interference at the early events of its replication.

Anticarcinogenic activity: Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7,12-dimethylbenz[a]anthracene (DMBA), as revealed by reduced incidence of neoplasm[88]. Neem may exert its chemopreventive effect in the oral mucosa by modulation of glutathione and its metabolizing enzymes. That neem leaf extract exerts its protective effect in *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (a carcinogenic material)-induced oxidative stress has also been demonstrated by the reduced formation of lipid peroxides and enhanced level of antioxidants and detoxifying enzymes in the stomach, a primary target organ for MNNG as well as in the liver and in circulation[89,90].

Hepatoprotective activity: The aqueous extract of neem leaf was found to offer protection against paracetamol-induced liver necrosis in rats[91]. The elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT) indicative of liver damage, were found to be significantly reduced on administration of the neem leaf aqueous extract.

Antioxidant activity: The antioxidant activity of neem seed extract has been demonstrated *in vivo* during horsegrain germination, which is associated with low levels of lipooxygenase activity and lipid peroxides[92]. An antioxidant principle has also been isolated, which is a potent inhibitor of plant lipooxygenases.

Effect on central nervous system: Varying degrees of central nervous system (CNS) depressant activity in mice was observed with the leaf extract[93]. Fractions of acetone extract of leaf showed significant CNS depressant activity[94]. Leaf extract up to a dose of 200 mg/kg body weight produces significant anxiolytic activity in rats[95]. The crude ethanolic extract of stem bark and root bark showed hypotensive, spasmolytic and diuretic activities[96,97].

Clinical studies and plausible medicinal applications of neem

Although a large number of studies have been carried out on various biological activities of neem extracts and some of the isolated compounds in several animal models, a few reports are available on clinical studies with the extracts or the compounds and their medicinal applications.

Neem extract

Clinical studies with the dried neem leaf extract indicated its effectiveness to cure ringworm, eczema and scabies. Lotion derived from neem leaf, when locally applied, can cure these dermatological diseases within 3–4 days in

acute stage or a fortnight in chronic case[98,99]. A paste prepared with neem and turmeric was found to be effective in the treatment of scabies in nearly 814 people[100]. In 97% of cases, the paste was found to cure scabies within 3–15 days of treatment without any adverse effect. Neem leaf extract has been prescribed for oral use for the treatment of malaria by Indian ayurvedic practitioners from time immemorial. Dried neem leaves in the form of tea are used by the people of Nigeria and Haiti to treat this disease[101]. Recently, a clinical trial has been carried out to see the efficacy of neem extract to control hyperlipidemia in a group of malarial patients severely infected with *P. falciparum*[102]. The lipid level, especially cholesterol, was found to be lower during therapy when compared to non-malaria patients. This is a report on malaria patients being treated with the neem extract on plasma lipid level during infection. Several clinical studies have been reported with the neem oil. Application of neem oil on the hair has been shown to kill head lice[101]. Reports are available regarding the use of neem to treat patients suffering from various forms of cancer[103]. One patient with parotid tumour and another with epidermoid carcinoma have responded successfully when treated with neem seed oil[104]. Although in trials neem oil has been shown to have antimicrobial effect to inhibit many species of pathogenic bacteria, including *S. aureus* and *Salmonella typhora*, it has not been considered as antibiotic due to some limitations[101]. Considerable clinical trials have been done on the antifertility effect of neem oil. NIM-76, a refined product from neem oil, was studied in 10 human volunteers, where intravaginal application before sexual intercourse could prevent pregnancy with no adverse effect on vagina, cervix and uterus[101]. After demonstrating the antifertility effect of intrauterine neem oil treatment (IUNT) in bonnet monkeys with no apparent side effects[71], phase I clinical trials were conducted on Praneem Vilci (PV)[105], a purified neem oil preparation on eighteen healthy tubectomized women to evaluate the safety after a single intrauterine instillation of PV and to determine the effects of its coadministration on anti-hCG response to the heterospecies dimer (HSD) hCG vaccine. Haematological and biochemical profiles, mid-luteal serum progesterone level and ovulatory status were determined before and after intrauterine treatment with PV. Except one woman showing nonspecific endometritis, no significant adverse effect was observed in other women and all women receiving PV and HSD vaccine produced antibodies against hCG. The data suggested that intrauterine treatment of PV is safe. The authors are unaware whether phase II and phase III clinical trials have been carried out. A polyherbal pessary (Praneem polyherbal pessary) has been developed using some purified ingredients from neem leaves, *Sapindus mukerossi* and *Mencitrata* oil, which shows spermicidal action *in vitro* on human sperm and *in vivo* on post-coital tests in women[106,107]. The formulation also has antimicrobial activity. Phase I clinical trials have been completed in India, Egypt and the Dominician Republic. These indicate the safety of its use with beneficial action in invaginosis due to microbial infection[106]. In most women, the pessary also prevented migration of sperm into the cervical mucus[107]. Praneem pessary has thus potential for the development of contraceptive devices.

Neem compound

There have been very few reports on the clinical trials done with bioactive compounds isolated from neem. Sodium nimbidinate, the sodium salt of nimbidin, the main bitter principle isolated from neem seed oil, has been shown to act as a potent diuretic under various clinical conditions[108]. In a limited clinical trial, oral administration of 100 mg nimbidin three times daily for 10 consecutive days to tropical eosinophilia patients, caused 25% reduction in total eosinophil count with a marked symptomatic relief[109].

Safety evaluation with various parts of neem and neem products

Various studies have been reported on the safety evaluation of different parts of neem as well as its various biologically active products[98,110–112]. As the details of these studies are beyond the scope of this review, only the major findings have been presented.

Neem bark

Neem stem bark extract shows lethal effect in three common snail species *Biomphalaria pfeifferi*, *Bulinus truncatus* and *Lymnaea natalensis* and against fish, *Aphyosemon giardneri*[113]. Methanolic extract of neem bark demonstrated oral LD_{50} at about 13 g/kg in acute toxicity studies on mice[98]. Detailed toxicity studies have recently been conducted in rats with the neem bark aqueous extract[114] as an extension of our studies showing antiacidsecretory–antiulcer activity of the bark extract[68]. In 14-day oral toxicity studies with 2 g/kg body weight, no lethal effect was observed, with no apparent change in relative organ weight, hematological parameters, enzyme levels and histopathology of several organs. The subacute (28 days) oral toxicity studies with both 0.05 and 0.1 g/kg body weight also revealed no abnormalities in the above-mentioned parameters. Acute and subacute (14 days) toxicity studies in the authors' laboratory on mice with 1 g/kg and 0.6 g/kg body weight respectively also showed no lethal effect with the bark extract.

Neem seed

Various neem seed preparations such as aqueous neem seed kernel extract demonstrated toxicity to *Oreochromis*

niloticus (tilapia) and *Cyprinus carpio* (carp)[110]. Nearly 60% mortality was observed in white leghorn chicks within a day of feeding powdered ripe neem berry aqueous extract[110,115]. Another feeding trial with neem seed meal (2.5%) on chicks[110] indicated mild to severe changes in kidney, liver, spleen, intestine and heart. An aqueous extract of neem seed kernel (1 ml/100 g body weight daily of a 50 g/l solution) produces trypsin inhibitory activity in weanling rats[110,116]. Retardation of spermatogenesis was observed by feeding neem seed cake to rats[110]. Calves fed with neem seed cake showed reduced haemoglobin content in the blood, along with depression[110].

Neem oil

Neem oil shows toxicity to fish like tilapia and carp, with an LC_{50} of 1124.6 and 302.7 ppm respectively[110]. Oral administration of neem oil at 200 mg/rat produces severe hypoglycaemic effect[110]. Neem seed oil showed acute toxicity in rats and rabbits with LD_{50} of 14 ml/kg and 24 ml/kg respectively, the possible target organs for toxic effects being the CNS and the lungs[117]. Neem seed oil produces toxic effect in humans in several isolated cases[98,110,118]. Neem oil intoxication by humans produces diarrhoea, nausea, vomiting, acidosis, encephalopathy, etc.[110,118]. These toxic effects might be due to presence of aflatoxin and other toxic compounds present in neem oil. Mechanistic investigations indicate that neem oil uncouples mitochondrial oxidative phosphorylation, thus inhibiting the respiratory chain. It also decreases intramitochondrial levels of acetyl CoA and acid-soluble CoA esters and reduces the mitochondrial ATP content[98,110,119].

Neem leaves

Methanolic extract of neem leaf exhibits oral toxicity in mice[98], showing signs of ill health and discomfort, gastrointestinal spasms, apathy, hypothermia and terminal convulsions, leading to death. Intravenously administered aqueous leaf extract at a dose greater than 40 mg/kg body weight produces toxic manifestation leading to death in guinea pigs[98]. Successive doses of 5–200 mg/kg reduces heart rate and increased the arterial pulse rate in guinea pigs[110]. Aqueous leaf extract also shows antifertility effect in mice when given through the oral route[73,98].

Table 3. Safety evaluation of different parts of neem

Part	Toxic/adverse effect	Animal in which toxicity is manifested	Reference
Bark	Lethal toxicity	Snail and fish	113
		Mice	98
Seed	Lethal toxicity	Tilapia and carp	110
		White leghorn chicks	110, 115
	Mild to severe changes in kidney, liver, spleen, intestine and heart	Chick	110
	Trypsin inhibitory activity	Weaning rat	110, 116
	Antispermatogenesis	Rat	110
	Reduced haemoglobin content and growth depression	Calf	110
Oil	Lethal toxicity	Tilapia and carp	110
	Acute toxicity	Rat, rabbit	117
	Severe hypoglycaemia	Rat	24, 64, 110
	Produces vomiting, diarrhoea, drowsiness, acidosis, encephalopathy	Human	98, 110, 118
	Spermicidal	Rhesus monkey and human	69
	Antifertility	Baboons and monkey	74
		Rodent	75
Leaf	Lethal toxicity	Mice, guinea pig	98
	Reduced heart rate and increased pulse rate	Guinea pig	110
	CNS-depressant	Mice	93
	Hepatonephropathy	Hisex chick	120
	Genotoxicity	Mice	121
	Antifertility	Mice	73, 98
	Decreased sperm count and motility	Rat	122
	Antiandrogenic	Rat	123
	Hypoglycaemia	Rat, rabbit	62, 64

Brown hisex chicks, when fed with a diet containing 2% and 5% neem leaf from their 7th to 35th day after birth developed hepatonephropathy and significant change in blood parameters[120]. Crude neem leaf extract causes genotoxicity in male mice germ cell at a dose of 0.5–2 g/kg body weight for 6 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[121]. Neem leaf extract when administered for 48 days in albino rats causes decrease in sperm count, sperm motility and forward velocity, probably due to androgen deficiency[122]. Oral administration of 20–60 mg dry leaf powder for 24 days in rats causes decrease in the weight of seminal vesicle and ventral prostrate, and regressive changes of the histological parameters through its antiandrogenic property[123]. Some toxicological manifestations of various parts of neem have been presented in Table 3.

Safety evaluation of neem compounds and marketed formulations

Nimbidin produces sub-acute toxicity in adult rats after daily administration of 25, 50 or 100 mg/kg for six weeks[98]. A significant hypoglycaemic effect was observed by feeding nimbidin to fasting rabbits[24]. Nimbidin also has spermicidal activity[28,29]. Nimbolide, a major chemical component of neem seed oil, and nimbic acid were found to be toxic to mice when given intravenously or intraperitoneally[110,124]. They are, however, less toxic to rats and hamster[124]. Nimbolide and nimbic acid at a lethal dose cause death in most animals by dysfunction of kidney, small intestine and liver as well as by marked and sudden drop of arterial blood pressure. Nimbolide shows potent cytotoxic effect[125] on N1E-115 neuroblastoma (mouse), 143B.TK-osteosarcoma (human) and Sf9 (insect) cultured cell lines with IC_{50} value of 4–10 μM. Other limonoids like epoxyazadiradione and salanin show cytotoxic effect at IC_{50} value of 27 and 112 μM respectively. Nimbidin, deacetylnimbin and azadirachtin are practically nontoxic. Acetylcholinesterase (AchE), Na^+–K^+, and Ca^{++}–ATPase are significantly inhibited, while Mg^{2+}–ATPase level increases significantly in rat brain when treated orally with 80, 160 and 320 mg/kg of vepacide, an active ingredient from neem seed oil, daily for 90 days[126]. Several studies were performed with Margosan 'O', an extract of neem seeds. However, no apparent toxic manifestations were noticeable in rats or mice[110]. LC_{50} of Margosan 'O' is more than 2 ml/kg in albino rabbits when tested for acute dermal toxicity[98]. However, Margosan 'O' showed minimal irritation in both eyes[98] when applied to one washed and one unwashed eye of albino rabbits over seven days. NIM-76, a volatile fraction of neem oil, possesses antifertility activity when applied before coitus in rats, rabbits and rhesus monkeys[127].

Conclusion

Neem, the versatile medicinal plant is the unique source of various types of compounds having diverse chemical structure. Very little work has been done on the biological activity and plausible medicinal applications of these compounds and hence extensive investigation is needed to exploit their therapeutic utility to combat diseases. A drug-development programme should be undertaken to develop modern drugs with the compounds isolated from neem. Although crude extracts from various parts of neem have medicinal applications from time immemorial, modern drugs can be developed after extensive investigation of its bioactivity, mechanism of action, pharmacotherapeutics, toxicity and after proper standardization and clinical trials. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from neem should be emphasized for the control of various diseases. In fact, time has come to make good use of centuries-old knowledge on neem through modern approaches of drug development. For the last few years, there has been an increasing trend and awareness in neem research. Quite a significant amount of research has already been carried out during the past few decades in exploring the chemistry of different parts of neem. Several therapeutically and industrially useful preparations and compounds have also been marketed, which generates enough encouragement among the scientists in exploring more information about this medicinal plant. An extensive research and development work should be undertaken on neem and its products for their better economic and therapeutic utilization.

1. Chopra, R. N., Nayer, S. L. and Chopra, I. C., *Glossary of Indian Medicinal Plants*, CSIR, New Delhi, 1956.
2. Chopra, R. N., Chopra, I. C, Handa, K. L. and Kapur, L. D. (eds), *Indigenous Drugs of India*, U.N. Dhur and Sons, Kolkata, 1958, pp. 51–595.
3. Kirtikar, K. R. and Basu, B. D., in *Medicinal Plants* (eds Blatter, E., Cains, J. F., Mhaskar, K. S.), Vivek Vihar, New Delhi, 1975, p. 536.
4. Thakur, R. S., Singh, S. B. and Goswami, A., *Curr. Res. Med. Aromat. Plants*, 1981, **3**, 135–140.
5. Koul, O., Isman, M. B. and Ketkar, C. M., *Can. J. Bot.*, 1990, **68**, 1–11.
6. Chatterjee, A. and Pakrashi, S. (eds), *The Treatise on Indian Medicinal Plants*, 1994, vol. 3, p. 76.
7. Schmutterer, H. (ed.), *The Neem Tree: Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes*, VCH, Weinheim, Germany, 1995, pp. 1–696.
8. Singh, R. P., Chari, M. S., Raheja, A. K. and Kraus, W., *Neem and Environment*, Oxford & IBH Publishing, New Delhi, 1996, Vols I and II, pp. 1–1198.
9. De Jussieu, A., *Mem. Mus. Hist. Nat., Paris*, 1830, **19**, 220.
10. Pennington, T. D. and Styles, B. T., *Blumea*, 1975, **22**, 419–540.
11. Pennington, T. D., *Flora Neotropica*, New York Botanical Garden, NY, Monogr. No. 28, 1981.
12. Siddiqui, S., *Curr. Sci.*, 1942, **11**, 278–279.

13. Mitra, C. R., *Neem*, Dr M. S. Patel, Indian Central Oilseeds Committee, Hyderabad, 1963, pp. 69–94.
14. Warthen, J. D. Jr., US Dept. of Agric. Sci. and Educ. Admin. Agric. Rev. and Man., ARM-NE-4, 1979, pp. 1–21.
15. Taylor, D. A. H., *Prog. Chem. Org. Nat. Prod.*, 1984, **45**, 1–101.
16. Champagne, D. E., Koul, O., Isman, M. B., Scudder, G. G. E. and Towers, G. H. N., *Phytochemistry*, 1992, **31**, 377–394.
17. Kraus, W., in *The Neem Tree: Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes* (ed. Schmutterer, H.), 1995, pp 35–88.
18. Devakumar, C. and SukhDev, in *Neem* (eds Randhawa and Parmar, B. S.), 1996, 2nd edn, pp. 77–110.
19. Govindachari, T. R., *Curr. Sci.*, 1992, **63**, 117–122.
20. Mitra, C. R., Garg, H. S. and Pandey, G. N., *Phytochemistry*, 1971, **10**, 857–864.
21. Bhargava, K. P., Gupta, M. B., Gupta, G. P. and Mitra, C. R., *Indian J. Med. Res.*, 1970, **58**, 724–730.
22. Pillai, N. R. and Santhakumari, G., *Planta Med.*, 1981, **43**, 59–63.
23. David, S. N., *Mediscope*, 1969, **12**, 25–27.
24. Pillai, N. R. and Santhakumari, G., *Indian J. Med. Res.*, 1981, **74**, 931–933.
25. Pillai, N. R. and Santhakumari, G., *Planta Med.*, 1984, **50**, 143–146.
26. Pillai, N. R., Seshadri, D. S. and Santhakumari, G., *Indian J. Med. Res.*, 1978, **68**, 169–175.
27. Pillai, N. R. and Santhakumari, G., *Ancient Sci. Life*, 1985, **5**, 91–97.
28. Sharma, V. N. and Saksena, K. P., *Indian J. Med. Res.*, 1959, **13**, 1038.
29. Sharma, V. N. and Saksena, K. P., *ibid*, 1959, **47**, 322.
30. Murthy, S. P. and Sirsi, M., *Indian J. Physiol. Pharmacol.*, 1958, **2**, 387–396.
31. Bhide, N. K., Mehta, D. J. and Lewis, R. A., *Indian J. Med. Sci.*, 1958, **12**, 141–145.
32. Rochanakij, S., Thebtaranonth, Y., Yenjal, C. H. and Yuthavong, Y., *Southeast Asian J. Trop. Med. Public Health*, 1985, **16**, 66–72.
33. Khalid, S. A., Duddect, H. and Gonzalez-Sierra, M. J., *J. Nat. Prod.*, 1989, **52**, 922–927.
34. Rojanapo, W., Suwanno, S., Somaree, R., Glinsukon, T. and Thebtaranonth, Y., *J. Sci. Thailand*, 1985, **11**, 177–188.
35. Rao, B. S., Nazma and Rao, J. M., *Curr. Sci.*, 1977, **46**, 714–716.
36. Butterworth, J. H. and Morgan, E. D., *J. Chem. Soc. Chem. Commun.*, 1968, 23–24.
37. Jones, I., Ley, S. V., Denholm, A. A., Lovell, H., Wood, A. and Sinden, R. E., *FEMS Microbiol. Lett.*, 1994, **120**, 267–273.
38. Van der Nat, J. M., Van der Sluis, W. G., 't Hart, L. A., Van Disk, H., de Silva, K. T. D. and Labadie, R. P., *Planta Med.*, 1991, **57**, 65–68.
39. Ara, I., Siddiqui, B. S., Faizi, S. and Siddiqui, S., *J. Chem. Soc., Perkin Trans.*, 1989, **I**, 343–345.
40. Pant, N., Garg, H. S., Madhusudanan, K. P. and Bhakuni, D. S., *Fitoterapia*, 1986, **57**, 302–304.
41. Kakai Tokkyo Koho, J. P., *Chem. Abstr.*, 1984, **100**, 91350.
42. Fujiwara, T., Takeda, T., Ogihara, Y., Shimizu, M., Nomura, T. and Tomita, Y., *Chem. Pharm. Bull.*, 1982, **30**, 4025–4030.
43. Fujiwara, T., Sugishita, E., Takeda, T., Ogihara, Y., Shimizu, M., Nomura, T. and Tomita, Y., *ibid*, 1984, **32**, 1385–1391.
44. Vander Nat, J. M., Kierx, J. P. A. M., Van Dijk, H., De Silva, K. T. D. and Labadie, R. P., *J. Ethnopharmacol.*, 1987, **19**, 125–131.
45. Vander Nat, J. M., Hart, L. A. T., Vander Sluis, W. G., Van Dijk, H., Vander Berg, A. J. J., De Silva, K. T. D. and Labadie, R. P., *ibid*, 1989, **27**, 15–24.
46. Moursi, S. A. H. and Al-Khatib, I. M., *Jpn J. Pharmacol.*, 1984, **36**, 527–533.
47. Dymock, *Pharmacogr. Ind.*, 1890, **1**, 324.
48. Varma, G. S., *Miracles of Neem Tree*, Rasayan Pharmacy, New Delhi, 1976.
49. Kirtikar, K. R. and Basu, B. D., in *Indian Medicinal Plants*, Lalitha Mohan Basu, Allahabad, 1935, 2nd edn, p. 536.
50. Ketkar, A. Y. and Ketkar, C. M., in *The Neem Tree*: *Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes* (ed. Schmutterer, H.), 1995, pp. 518–525.
51. Tidjani, M. A., Dupont, C. and Wepierre, J., *Planta Med. Phytother.*, 1989, **23**, 259–266.
52. Lorenz, H. K. P., *J. Praxis*, 1976, **8**, 231–233.
53. Murthy, S. P. and Sirsi, M., *Indian J. Physiol. Pharmacol.*, 1958, **2**, 456–460.
54. Okpanyi, S. N. and Ezeukwv., G. C., *Planta Med.*, 1981, **41**, 34–39.
55. Vohra, S. B. and Dandiya, P. C., *Fitoterapia*, 1992, **63**, 195–207.
56. Jacobson, M., *Neem Newsl.*, 1986, **3**, 39–43.
57. Njiro, S. M. and Kafi-Tsekpo, M. W., *Ondersterpoort J. Vet. Res.*, 1999, **66**, 59–62.
58. Sen, P., Medinata, P. K. and Ray, A., *Indian J. Exp. Biol.*, 1992, **12**, 1170–1175.
59. Ray, A., Banerjee, B. D. and Sen, P., *ibid*, 1996, **34**, 698–701.
60. Upadhyay, S. N., Dhawan, S., Garg, S. and Talwar, G. P., *Int. J. Immunopharmacol.*, 1992, **14**, 1187–1193.
61. Murty, K. S., Rao, D. N., Rao, D. K. and Murty, L. B. G., *Indian J. Pharmacol.*, 1978, **10**, 247–250.
62. El-Hawary, Z. M. and Kholief, T. S., *Arch. Pharmacol. Res.*, 1990, **13**, 108–112.
63. Chakraborty, T., Uerotta, L. and Poddar, G., *Phytother. Res.*, 1989, **3**, 30–32.
64. Khosla, P., Bhanwra, S., Singh, J., Seth, S. and Srivastava, R. K., *Indian J. Physiol. Pharmacol.*, 2000, **44**, 69–74.
65. Chattopadhyay, R. R., *Gen. Pharmacol.*, 1996, **27**, 431–434.
66. Chattopadhyay, R. R., *J. Ethnopharmacol.*, 1999, **67**, 373–376.
67. Garg, G. P., Nigam, S. K. and Ogle, C. W., *Planta Med.*, 1993, **59**, 215–217.
68. Bandyopadhyay, U., Chatterjee, R. and Bandyopadhyay, R., US Patent 5,730,986, 1998; corresponding to Indian Patent 1100/Del/95.
69. Sinha, K. C. *et al.*, *Indian J. Med. Res.*, 1984, **79**, 131–136.
70. Upadhyay, S. N., Kaushic, C. and Talwar, G. P., *Proc. R. Soc. London B*, 1990, **242**, 175–179.
71. Upadhyay, S., Dhawan, S., Sharma, M. G. and Talwar, G. P. *Contraception*, 1994, **49**, 161–169.
72. Kaushic, C. and Upadhyay, S., *ibid*, 1995, **51**, 203–207.
73. Despande, V. Y., Mendulkar, K. N. and Sadre, N. L., *J. Postgrad. Med.* (*Bombay*), 1980, **26**, 167–170.
74. Mukherjee, S., Lohiya, N. K., Pal, R., Sharma, M. G. and Talwar, G. P., *Contraception*, 1996, **53**, 375–378.
75. Mukherjee, S., Garg, S. and Talwar, G. P., *J. Ethnopharmacol.*, 1999, **67**, 287–296.
76. Khalid, S. A., Farouk, A., Geary, T. G. and Jensen, J. B., *ibid*, 1986, **15**, 201–209.
77. Badani, L., Deolankar, R. P., Kulkarni, M. M., Nagsampgi, B. A. and Wagh, U. V., *Indian J. Malariol.*, 1987, **24**, 111–117.
78. Dhar, R., Zhang, K., Talwar, G. P., Garg, S. and Kumar, N., *J. Ethnopharmacol.*, 1998, **61**, 31–39.
79. Khan, M. and Wassilew, S. W., in *Natural Pesticides from the Neem Tree and Other Tropical Plants* (eds Schmutterer, H. and Asher, K. R. S.), GTZ, Eschborn, Germany, 1987, pp. 645–650.
80. Chopra, I. C., Gupta, K. C. and Nair, B. N., *Indian J. Med. Res.*, 1952, **40**, 511–515.
81. Satyavati, G. V., Raina, M. K. and Sharma, M. (eds), *Medicinal Plants of India*, 1976, vol. I.
82. Almas, K., *Indian J. Dent. Res.*, 1999, **10**, 23–26.
83. SaiRam, M. *et al.*, *J. Ethnopharmacol.*, 2000, **71**, 377–382.

84. Baswa, M., Rath, C. C., Dash, S. K. and Mishra, R. K., *Microbios.*, 2001, **105**, 183–189.
85. Rao, A. R., Kumar, S., Paramsivam, T. B., Kamalakshi, S., Parashuram, A. R. and Shantha, M., *Indian J. Med. Res.*, 1969, **57**, 495–502.
86. Gogati, S. S. and Marathe, A. D., *J. Res. Educ. Indian Med.*, 1989, **8**, 1–5.
87. Badam, L., Joshi, S. P. and Bedekar, S. S., *J. Commun. Dis.*, 1999, **31**, 79–90.
88. Balasenthil, S., Arivazhagan, S., Ramachandran, C. R. and Nagini, S., *J. Ethnopharmacol.*, 1999, **67**, 189–195.
89. Arivazhagan, S., Balasenthil, S. and Nagini, S., *Cell Biochem. Funct.*, 2000, **18**, 17–21.
90. Arivazhagan, S., Balasenthil, S. and Nagini, S., *Phytother. Res.*, 2000, **14**, 291–293.
91. Bhanwra, S., Singh, J. and Khosla, P., *Indian J. Physiol. Pharmacol.*, 2000, **44**, 64–68.
92. Rao, A. D., Devi, K. N. and Thyagaraju, K., *J. Enzyme Inhib.*, 1998, **14**, 85–86.
93. Singh, S. D., Junnarkar, A. Y., Reddi, G. S. and Singh, K. V., *Fitoterapia*, 1987, **58**, 235–238.
94. Singh, P. P., Junnarkar, A. Y.,Thomas, G. P., Tripathi, R. M. and Varma, R. K., *ibid*, 1980, **61**, 164–168.
95. Jaiswal A. K., Bhattacharya, S. K. and Acharya, S. B., *Indian J. Exp. Biol.*, 1994, **32**, 489–491.
96. Bhakuni, D. S., Dhar, M. L., Dhar, M. M., Dhawan, B. N., Gupta, B. and Srimal, R. C., *ibid*, 1971, **9**, 91–102.
97. Abraham, Z., Bhakuni, D. S., Garg, H. S., Goel, A. K., Mehrotra, B. N. and Patnaik, G. K., *ibid*, 1986, **24**, 48–68.
98. Kanungo, D., in *Neem* (eds Randhawa and Parmar, B. S.), 1996, 2nd edn, pp. 77–110.
99. Anonymous, Doctors Find Neem Good for Skin Diseases, *New Delhi Evening News*, 1985.
100. Charles, V. and Charles, S. X., *Trop. Geogr. Med.*, 1992, **44**, 178–181.
101. Report, Board on Science and Technology for International Development, National Research Council, National Academy Press, Washington DC, 1992, pp. 60–113.
102. Njoku, O. U., Alumanah, E. O. and Meremikwu, C. U., *Boll. Chim. Farm.*, 2001, **140**, 367–370.
103. Hartwell, J. L., *Quarterman Lawrence Mass.*, 1982, **33**, 181.
104. Chatterjee, K. K., *Indian Med. Rec.* (*Calcutta*), 1961, **81**, 101.
105. Talwar, G. P. *et al.*, *Indian J. Med. Res.*, 1995, **102**, 66–70.
106. Talwar, G. P., Raghuvanshi, P., Misra, R., Mukherjee, S. and Shah, S., *Immunol. Cell Biol.*, 1997, **75**, 190–192.
107. Raghuvanshi, P., Bagg, R., Malhotra, D., Gopalan, S. and Talwar, G. P., *Indian J. Med. Res.*, 2001, **113**, 135–141.
108. Shah, M. P., Seth, U. K., Bhide, N. K. and Shah, M. J., *Indian J. Med. Sci.*, 1958, **12**, 150.
109. Gopinathan, G., MD Thesis, University of Kerala, 1973.
110. Jacobson, M., in *The Neem Tree*: *Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes* (ed. Schmutterer, H.), 1995, pp. 484–495.
111. Ali, B. H., *J. Ethnopharmacol.*, 1992, **35**, 267–273.
112. Van der Nat, J. M., Van der Sluis, W. G., de Silva, K. T. and Labadie, R. P., *ibid*, 1991, **35**, 1–24.
113. Osuala, F. O. and Okwuosa, V. N., *Appl. Parasitol.*, 1993, **34**, 63–68.
114. Project Code No. SSP-60, Industrial Toxicology Research Center, Council of Scientific and Industrial Research, Lucknow, 1999.
115. Singh, Y. P., Bahga, H. S. and Vijjan, V. K., *Neem Newsl.*, 1985, **2**, 17.
116. Rao, P. U., *J. Am. Oil Chem. Soc.*, 1987, **64**, 1348–1351.
117. Gandhi, M., Lal, R., Sankaranarayanan, A., Banerjee, C. K. and Sharma, P. L., *J. Ethnopharmacol.*, 1988, **23**, 39–51.
118. Sinniah, D. and Baskaran, G., *Lancet*, 1981, **28**, 487–489.
119. Koga, Y., Yoshida, I., Kimura, A., Yoshino, M., Yamashita, F. and Sinniah, D., *Paediatr. Res.*, 1987, **22**, 184–187.
120. Ibrahim, I. A., Khalid, S. A., Omer, S. A. and Adam, S. E., *J. Ethnopharmacol.*, 1992, **35**, 267–273.
121. Awasthy, K. S., *Cytobios*, 2001, **106**, 151–164.
122. Aladakatti, R. H., Nazeer Ahmed, R., Ahmed, M. and Ghosewami, M. G., *J. Basic Clin. Physiol. Pharmacol.*, 2001, **12**, 69–76.
123. Kasutri, M., Ahmed, R. N., Pathan, K. M., Shaikh, P. D. and Manivannan, B., *Indian J. Physiol. Pharmacol.*, 1997, **41**, 234–240.
124. Glinsukon, T., Somjaree, R., Piyachaturawat, P. and Thebtaranonth, Y., *Toxicol. Lett.*, 1986, **30**, 159–166.
125. Cohen, E., Quistad, G. B. and Casida, J. E., *Life Sci.*, 1996, **58**, 1075–1081.
126. Rahman, M. F., Siddiqui, M. K. and Jamil, K., *J. Environ. Sci. Health B.*, 1999, **34**, 873–884.
127. Riar, S. S. *et al.*, *Contraception*, 1991, **44**, 319–326.

Received 22 October 2001; revised accepted 20 February 2002