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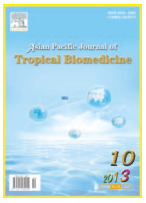

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Review on pharmacological and toxicologyical effects of oleum azadirachti oil

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PEER REVIEW

Peer reviewer

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Comments

This is a valuable review work in which author has demonstrated the pharmacological, toxicological, preparation, botanical investigations of oleum azadirachti oil. Oleum azadirachti oil was found to be a promising to be usefulness in pharmacological industry.
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ABSTRACT

Oleum azadirachti consists of the oil obtained from dried seeds of *Azadirachta indica* A. Juss. (family: Meliaceae). Local names of *Azadirachta indica* A. Juss. are Abodua, aforo-oyinbo, anwe egyane, arista, azad dirakht, azadarakht, azedarach and bead tree. Indigenous to India, and widely distributed in South and South-East Asia and cultivated in Africa, the South Pacific Islands, South and Central America and Australia, and in southern Florida and California, United States of America, it is a straight-boled deciduous tree, which is 6-25 m high. Bark is dark-brown, externally fissured with a buff inner surface and fibrous fracture. Leaves alternately arranged, pinnately compound and up to 40 cm long, and composed of 8-18 short-petiolate narrow-ovate, pointed and curved toothed leaflets, 3-10 cm long and 1-4 cm wide arranged in alternate pairs. The major constituents are oxidized tetranortriterpenes including azadirachtin (azadirachtin A), azadiriadione, epoxyazadiradione, azadirone, nimbidin, nimbin, deacetylnimbin, salannin, gedunin, mahmoodin, 17-hydroxydiradione and related derivatives. It is of various medicinal uses, such as a contraceptive for intravaginal use, a mosquito repellent, and treatment of vaginal infections, treatment of gastric ulcers, cardiovascular disease, malaria, rheumatism and skin disorders, external applications for treatment of septic wounds,ulcers and boils, treatment of allergic skin reactions, asthma, bruises, colic, conjunctivitis, dysmenorrhoea, fever, gout, headache, itching due to varicella, kidney stones, leukorrhoea, psoriasis, scabies, sprains and muscular pain, and wounds. It is also used as an emmenagogue, tonic, stomatic and vermicide. In conclusion, the plant oil had antifertility, antihyperglycaemic, anti-inflammatory, antimicrobial, antiviral, antiulcer, estrogenic, immune, contraceptive, antibacterial, insect repellent, and skin treatment effects.

KEYWORDS

Oleum azadirachti; *Azadirachta indica*, Meliaceae, Pharmacology, Toxicology

1. Introduction

Oleum azadirachti consists of the oil obtained from dried seeds of *Azadirachta indica* A. Juss. (family: Meliaceae) (*A. indica*). Local names of *A. indica* are Abodua, aforo-oyinbo, anwe egyane, arista, azad dirakht, azadarakht, azedarach, bead tree, bevinama, bevu, bewina mara, bodetso, bo-nim, cape lilac, chajara hourra, chichaâne arbi, China berry, China tree, cót anh, darbejiya, dogo yaro, dogón yaro, dogonyaro, dogoyaro, dongo yaro, dua gyane, gori, gringging, holy tree, igi-oba, imba, Indian lilac, Indian lilac tree, Indian neem tree, Indian sadao, Intaran, isa-bevu, jaroud, kahibevu, kingtsho, kiswahhili, kohhomba, kohumba, koummar, kuman masar, kuman nasara, kwinin, labkh, lilac de perse, lilas des indes, liliti, limb, limba, limbado, limado, linigbe, mahanim, mahanimba, mahnimu, mak tong, margosa, margosa tree, margose, marrar, mimba, mindi, miro tahiti, mwarobaini,

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neeb, neem, neem sikha, nim, nim tree, nimba, nimbatikta, nimgach, nivaquine, ogwu akom, oilevevu, ouchi, Persian lilac, phãk kã dão, picumarda, sa-dao, sa-dao baan, sadao India, sdau, salien, sandan, sandannoki, sãu dâu, senjed talhk, shajarat el horrah, shereesh, tâak, tâakhak, touchenboku, vembu, vemmu, vepa, veppam, veppu, white cedar, xoan dào, zanzalakht and zaytoon[1-9]. Indigenous to India, and widely distributed in South and South-East Asia, cultivated in Africa, the South Pacific Islands, South and Central America and Australia, and in southern Florida and California, United States of America[1-3,7,10,11], it is a straight-boled deciduous tree, which is 6-25 m high. Bark is dark-brown, externally fissured with a buff inner surface and fibrous fracture. Leaves are alternately arranged, pinnately compound, up to 40 cm long, composed of 8-18 short-petiolate narrow-ovate, pointed, curved toothed leaflets, 3-10 cm long and 1-4 cm wide arranged in alternate pairs. Inflorescences are axillary panicles; flowers are numerous, white, pedicellate and about 1.0 cm wide. Fruits are yellowish drupes, oblong and about 1.5 cm long, containing thin pulp surrounding a single seed. When bruised, leaves and twigs emit an onion-like odour[1-3,7,11]. The major constituents are oxidized tetranortriterpenes including azadirachtin (azadirachtin A), azadiriadione, epoxyazadiradione, azadirone, nimbidin, nimbin, deacetylnimbin, salannin, gedunin, mahmoodin, 17-hydroxydiradione and related derivatives[9,11-16]. The structures of azadirachtin, nimbin and deacetylnimbin are presented in Figure 1. It is of various medicinal uses, such as a contraceptive for intravaginal use[17], a mosquito repellent[18,19], treatment of vaginal infections[20], gastric ulcers, cardiovascular disease, malaria, rheumatism and skin disorders, external applications for treatment of septic wounds, ulcers and boils[7], treatment of allergic skin reactions, asthma, bruises, colic, conjunctivitis, dysmenorrhoea, fever, gout, headache, itching due to varicella, kidney stones, leukorrhoea, psoriasis, scabies, sprains and muscular pain, and wounds[10,11]. It is also used as an emmenagogue, tonic, stomatic and vermicide[9].

This review should act to stimulate a thought process on the importance of pharmacological effect of oleum azadirachti oil and the usefulness of this plant oil in pharmaceutical industry process.

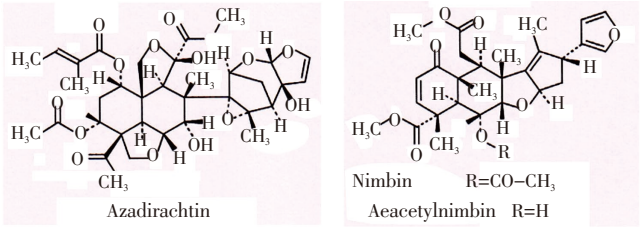

Figure 1. The structures of azadirachtin and deacetylnimbin.

2. Preparation of oleum azadirachti

A. indica seeds were air-dried in an oven at 40 °C for 4 d and then the dried seeds were crushed, pulverized, and weighed in sequence to oil preparation. Distilled water was placed in the steam generator and heated to produce steam. The seed powder was placed in the round bottom flask. A vigorous current of steam from steam generator was passed through the round bottom flask, and a part of the steam condensed in the round bottom flask. As more and more steam was passed, the steam volatile components of the seed powder passed through the condenser along with the steam. These contents in condensation are collected in the receiver. The contents in the round bottom flask were heated by a Bunsen burner to prevent excessive condensation of the steam. The process of steam distillation was continued for about half an hour. The distillate was transferred to a separating funnel and extract with petroleum ether for 3 times. Then, the petroleum ether extract was dried with anhydrous sodium sulphate. The solvent was removed from the dried filtrate by careful distillation in a water bath and the essential oil is left behind in the distillation flask.

3. Toxicology

Studies of the oral acute toxicity of the oil in rats and rabbits showed dose-related pharmacotoxic symptoms along with a number of biochemical and histopathological indices of toxicity. The 24-hour oral median lethal dose was 14 mL/kg body weight in rats and 24 mL/kg body weight in rabbits. Prior to death, all animals exhibited pharmacotoxic symptoms of a similar type and severity; the lungs and central nervous system were the target organs[21]. Intragastric administration of the oil to mice was not toxic at a dose of 2 mL. The oil was nonirritant when applied to the skin of rabbits in a primary dermal irritation test. In a subacute dermal toxicity study, rabbits exposed to the oil daily for 21 days showed no significant changes in body weight or organ: body weight ratio, serum oxaloacetic transaminase and pyruvic transaminase levels, and blood glucose and urea nitrogen values. No treatment-related histopathological changes were observed[22]. In a three-generation study carried out according to World Health Organization/United States Food and Drug Administration protocol, groups of 15 male and 15 female rats were fed a diet containing 10% oleum azadirachti or peanut oil. Reproductive toxicology was monitored for three generations. There were no adverse effects on the reproductive parameters in either group[23]. A group of 10 pregnant rats received 2 mL/kg body weight of the oil by

gastric administration daily and the animals were allowed to deliver at term. Six of the treated animals died between days 6 and 13 of pregnancy. Among the four remaining animals that delivered, one delivered a seemingly normal rat on day 27, but the rat died after 4 d. Autopsy performed on day 16 of pregnancy suggested that fetal resorption had occurred; however, no indication was given as to whether fetuses were normal[24]. Furthermore, The product Azatin, composed of 3% azadirachtin, also exhibited high toxicity against third instars of the scarab larvae [*Popillia japonica* (LC_{50}=1.13 mL/L), *Rhizotrogus majalis* (LC_{50}=0.81 mL/L), and *Anomala orientalis* (LC_{50}=1.87 mL/L][25]. The estimated safe dose of azadirachtin=15 mg/kg body weight/day[26].

4. Pharmacological effects

4.1. Antifertility effect

Oleum azadirachti (0.6 mL) was given to female rats by intragastric administration on days 8-10 of pregnancy after confirming the presence and number of embryo implants surgically on Day 7. The animals were examined again under anaesthesia on Day 15 of pregnancy to check the number of developing embryos. Controls received an equivalent regime of peanut oil. Complete resorption of embryos was observed on Day 15 of pregnancy in every animal treated with oleum azadirachti while embryos were developing normally in controls[27]. Intragastric administration of 6 mL of the oil per day for 60 d to female baboons induced abortion in pregnant animals[28]. A single intrauterine application of 100 μL of the oil produced a reversible block in fertility lasting for 107-180 d in female rats and 7-11 months in monkeys[29,30]. In an attempt to find an alternative to vasectomy for long-term male contraception, the effect of a single intra-vas application of the oil was assessed in male rats. Animals with proven fertility were given a single dose of 50 μL of the oil in the lumen of the vas deferens on each side. Control animals received the same volume of peanut oil. Animals were allowed free access to mating with females of proven fertility for 4 weeks after the treatment. While the control animals impregnated their female partners, all males were treated with oleum azadirachti remained infertile throughout the 8-month observation period. Epididymal and vas histologies were normal with no inflammatory changes or obstruction. Administration of the oil resulted in a block of spermatogenesis without affecting testosterone production. The seminiferous tubules, although reduced in diameter, appeared normal and contained mostly early spermatogenic cells. No anti-sperm antibodies were detected in the serum[31]. Subcutaneous administration of up to 0.3 mL of the oil to rats had no estrogenic, anti-estrogenic or progestational activity, and appeared not to interfere with the action of progesterone[32]. Intravaginal application of 2.50 μL-0.25 mL of the oil to pregnant rats induced abortion[33]. The oil (10%-25%) inhibited fertilization in isolated mouse ova as assessed by sperm-egg interaction, and impaired the development of fertilized ova *in vitro*[34]. In other investigations, the active constituents of the oil were identified to be a mixture of six compounds comprising saturated, mono and di-unsaturated free fatty acids and their methyl esters[35]. The oil (0.25-25.00 mg/mL) had spermicidal effects on human and rat sperm *in vitro*[36,37].

4.2. Antihyperglycaemic effect

Intragastric administration of 21 mg/kg body weight of the oil reduced blood glucose levels in rats[38]. A significant (P<0.01) reduction in blood glucose levels was observed in normal and alloxan-induced diabetic rabbits after administration of 200 mg of the oil; the effect was more pronounced in diabetic animals[38,39].

4.3. Anti-inflammatory effect

The anti-inflammatory effect of nimbidin was assessed and compared with phenylbutazone. Intramuscular administration of 40 mg/kg body weight of nimbidin reduced acute paw oedema in rats induced by carrageenan and kaolin. Formalin-induced arthritis in ankle joints and fluid exudation due to granuloma induced by croton oil in rats were also suppressed by similar treatment with the compound. In the acute phase of inflammation, nimbidin at 40 mg/kg body weight was more active than phenylbutazone at 100 mg/kg body weight[40]. Intramuscular administration of 50 mg/kg body weight of the oil reduced granuloma induced by cotton pellet in rats[41].

4.4. Antimicrobial and antiviral effects

The efficacy of a petroleum ether extract of the oil was investigated for its antimicrobial activity against certain bacteria and fungi and poliovirus compared with the oil. The extract had stronger antimicrobial activity than the oil and, *in vitro* at 2 mg/mL, inhibited the growth of *Escherichia coli* and *Klebsiella pneumoniae*, which were not inhibited by the oil. The extract was active against *Candida albicans* (minimum inhibitory concentration 0.25 mg/mL) and had antiviral activity against poliovirus replication in Vero African green monkey kidney cell lines at 50 μg/mL[42]. Intravenous administration of 60 mg/kg body weight of the oil twice per day for 7 d protected mice from systemic candidiasis, as shown by enhanced survival and a reduction in colony-forming units of *Candida albicans* in various tissues[42]. The oil inhibited

the growth of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus pyogenes in vitro* at a concentration of 1.5%-6.0%[43]. A petroleum ether extract of the oil inhibited the growth of *Epidermophyton floccosum, Microsporum canis, Microsporum gypseum, Trichophyton concentricum, Trichophyton rubrum* and *Trichophyton violaceum*[44]. Simeone *et al*. revealed that azadirachtin had antifungicide which they detected by liquid chromatography[45].

4.5. Antiulcer effect

Intragastric administration of 40 mg/kg body weight of nimbidin showed antiulcer activity in various experimental models (gastric lesions induced by acetylsalicylate, stress, serotonin and indometacin) in rats. The compound also protected rodents against cysteamine-and histamine-induced duodenal lesions[46].

4.6. Estrogenic effect

Subcutaneous administration of 0.2-6.0 mL/kg body weight of the oil to normal or ovariectomized rats had no estrogenic effects: there was no increase in uterine wet weight or disruption of the estrous cycle[32,33].

4.7. Immune effect

Mice received oleum azadirachti (150 μL/animal) or an emulsifying agent, with or without peanut oil, by intraperitoneal injection. Peritoneal lavage on subsequent days showed an increase in the number of leukocytic cells on day 3 following treatment with oleum azadirachti, and peritoneal macrophages exhibited enhanced phagocytic activity and expression of major histocompatability complex class II antigens. Treatment also induced the production of γ-interferon. The spleen cells of oil-treated animals showed a significantly higher lymphocyte proliferative response to *in vitro* challenge with concanavalin A or tetanus toxin than those of controls. Pretreatment with the oil did not augment the anti-tetanus-toxin antibody response. The results of this study indicate that the oil acts as a nonspecific immunostimulant and that it selectively activates cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge[47]. Intraperitoneal administration of the oil to mice (150 μL/animal) and rats (120 μL/animal) enhanced phagocytosis of macrophages[47,48]. The oil obtained from *A. indica* plant had cytotoxic and proliferating activities; the oil significantly reduced the proliferating cell nuclear antigen levels in the cell and the apoptotic activity due to mitochondial pathway[49].

5. Clinical effects

5.1. Contraceptive effect

In an uncontrolled clinical trial involving 225 healthy fertile women aged 18-35 years performed to assess the efficacy of the oil as an antifertility agent, subjects were instructed to insert 1 mL of the oil into the vagina with a plastic applicator 5 min prior to coitus and no other contraception was used. After 16 months of use only three pregnancies due to drug failure were reported; there were 30 pregnancies due to noncompliance (*i.e.* women who did not use the oil as instructed)[20].

5.2. Antibacterial effect

In a 2-week double-blind, placebo-controlled clinical trial involving 55 women with abnormal vaginal discharge due to bacterial vaginosis, subjects were instructed to insert 5 mL of the oil or placebo oil into the vagina daily. Treatment with the test oil was reported to cure the symptoms of the infection[23].

5.3. Insect repellent effect

In a field study carried out to evaluate the mosquito repellent action of the oil in villages in a forested area in Mandla District, Madhya Pradesh, India, various concentrations of the oil were mixed with coconut oil (1%-4%) and applied to the exposed body parts of human volunteers. The mixture provided 81%-91% protection from the bites of anopheline mosquitoes during a 12-hour period of observation[21]. Moreover, azadirachtin is used as biological pesticide control to avoid damage to the crops; where the oil is used and nontoxic to the rove beetle (Atheta coriaria) adults, and biological control agent is mainly used against fungus gnats (*Bradysia* spp.)[50,51].

5.4. Skin treatment effect

In a case report study, administration of 100 mg of oil twice daily for 34 d completely healed chronic skin ulcers up to 1 cm deep[52].

5.5. Overdose side effect

A 60-year-old male was admitted to hospital with neurological and psychotic symptoms following ingestion of 60 mL of oleum azadirachti. However, correlation of the adverse effects with ingestion of the oil was not definitely proven[53]. Oral administration of oleum azadirachti is contraindicated during pregnancy, nursing and in children under the age of 12 years. The number of cases of toxicity,

including toxic encephalopathy, poisoning and Reye-like syndrome, following ingestion of excessive doses of oleum azadirachti has been reported[54-56].

6. Precautions

6.1. Drug interactions effect

Administration of the oil may reduce blood glucose levels. It should therefore be used with caution in insulin-dependent diabetic patients or patients taking oral antihyperglycaemic drugs[38,39].

6.2. Carcinogenesis and mutagenesis effects

An acetone extract of the oil was inactive at concentrations of up to 200 mg/plate in the *Salmonella*/microsome assay using *Salmonella typhimurium* strains TA98 and TA100[57]. In the same test, the oil was not mutagenic using *Salmonella typhimurium* strains TA98 and TA100, with or without metabolic activation[58].

6.3. Teratogenic effect

The oil had embryotoxic effects after vaginal administration to pregnant rats at a dose of 0.25 mL/animal[37,38]. Embryotoxic effects were also reported following intragastric administration of 4 mL/kg body weight of the oil to pregnant rats on days 6-8 of pregnancy[27].

6.4. Dose and storage

The dose of 1-5 mL of oil was for intravaginal applications[20,22]. It was stored in a tightly sealed container away from heat and light.

7. Conclusion

Oleum azadirachti consists of the oil obtained from dried seeds of *A. indica* (family: Meliaceae). The dose of 1-5 mL of oil is used for intravaginal applications. The plant oil had antifertility, antihyperglycaemic, anti-inflammatory, antimicrobial, antiviral, antiulcer, estrogenic, immune, contraceptive, antibacterial, insect repellent, and skin treatment effects.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Oleum azadirachti consists of the oil obtained from dried seeds of *A. indica* (family: Meliaceae). It is indigenous to India, and widely distributed in South and South-East Asia. Medicinal uses of the oil are as a contraceptive for intravaginal use, as a mosquito repellent, and for treatment of vaginal infections. Also, it is used for treatment of gastric ulcers, cardiovascular disease, malaria, rheumatism and skin disorders. Therefore there is a need to explore the pharmacological and toxicological effects of oleum azadirachti oil and these are of usefulness of this plant oil in pharmaceutical industry process and the development of new drugs.

Research frontiers

This review article reveals some experimental and clinical pharmacological activities of oleum azadirachti oil as well as its toxicological effects and summarized the botanical and geographical distribution of the plants all over the world.

Related reports

Oleum azadirachti oil has antifertility, antihyperglycaemic, anti-inflammatory, antimicrobial, antiulcer, estrogenic, immune effects in addition to its clinical, overdose side effects. This review resembles a thought about the importance of this oil to be used in the industry.

Innovations and breakthroughs

Oleum azadirachti oil is worldwide distributed and used in a folk medicine to treat many body disorders like gastric ulcers, cardiovascular disease, malaria and rheumatism. In this review, author focus on experimental and clinical pharmacology effects in addition to botanical, preparation as well as toxic side effect of the oil.

Applications

From the literature survey it has been found that oleum azadirachti oil has antifertility, antihyperglycaemic, anti-inflammatory, antiulcer, antimicrobial, estrogenic, immune, Insect repellent effects. This scientific review

supports and suggests the important use of this plant oil in pharmacological industry supported by its widely distribution all over the world of the plant.

Peer review

This is a valuable review work in which author has demonstrated the pharmacological, toxicological, preparation, botanical investigations of oleum azadirachti oil. Oleum azadirachti oil was found to be a promising to be usefulness in pharmacological industry.

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