REVIEW ON THE PHARMACOLOGICAL ACTIVITIES OF ANETHOLE

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ABSTRACT

In recent years there has been an increasing interest in the activities of phytopharmaceutical products and biologically active substances of plant origin. Anethole is such a substance used from ancient times in traditional medicine in many countries. Nowadays it is widely used in food and beverage industry. Its widespread use and accessible price justify carrying out extensive scientific research in order to support the traditional uses of anethole with scientific evidence. This review article summarizes the current knowledge of the traditional use of anethole, its pharmacological activities and the possible mechanisms underlying its effects.

Keywords: *anethole, physicochemical properties, pharmacokinetics, traditional use, pharmacological activities*

PHYSICOCHEMICAL PROPERTIES OF ANETHOLE

The chemical structure of anethole is given in Fig. 1. Anethole exists as both cis and trans isomers, involving the double bond outside the ring. The more abundant isomer, and the one preferred for use, is the trans or E isomer.

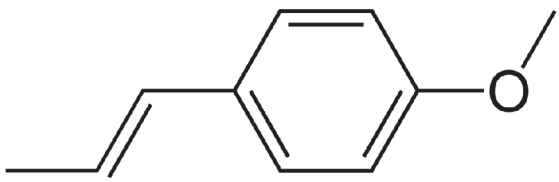

Fig. 1. Chemical structure of anethole

Anethole is clear, colorless to amber liquid with a sweet anise-like flavor. Anethole is only slightly soluble in water but exhibits high solubility in ethanol. This difference causes certain anise-flavored liqueurs to become opaque when diluted with water. This is due to the spontaneous formation of a microemulsion.

Anethole is distinctly sweet, 13 times sweeter than sugar. It is used in many alcoholic drinks. It flavors Middle Eastern arak, Colombian aguardiente, French spirits absinthe, anisette and pastis, Greek ouzo, Bulgarian and Macedonian mastika, German Jägermeister, Italian sambuca, Dutch Brokmöpke, Portuguese, Peruvian, and Spanish anísado, Herbs de Majorca, Mexican Xtabentún and Turkish rakı.

Anethole can cover unpleasant odours, so it is widely used as a masking agent in commodities, such as toilet soap, toothpaste, mouthwash, etc. This is the main use of anethole, about 80% of the total. It is used as a flavouring additive and scent in food industry products such as candy, baked goods, chewing gum, cigarettes, etc.

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OCCURRENCE IN NATURE

Anethole is a type of aromatic compound that occurs widely in nature in essential oils. It contributes to the distinctive flavors of anise and fennel (both in the botanical family Apiaceae), anise myrtle (Myrtaceae), liquorice (Fabaceae), camphor, magnolia blossoms, star anise (Illiciaceae) and many other plants. Natural anethole occurs in high concentrations in anise oil (80-90%), star anise oil (over 90%) and fennel oil (80%).

The essential oil in a plant has two types of function: protection and communication. It affords the host plant protection from pathogenic microorganisms such as viruses, bacteria and fungi, and/or deters herbivorous animals from consuming the plant. The "fragrant cloud" surrounding the plant may attract a particular species of bee, for example, that will help the plant reproduce by cross-pollination when it visits similar plants.

PHARMACOKINETICS OF ANETHOLE

Orally administered trans-anethole is rapidly absorbed, undergoes nearly complete metabolism in the liver producing metabolites that are conjugated and then excreted primarily in the urine. Some elimination as CO_2 in expired air also occurs.

TRADITIONAL USE OF ANETHOLE

The medicinal use of aniseed is largely due to antispasmodic, secretolytic, secretomotor and antibacterial effects of its essential oil. The traditional uses of aniseed for "dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, bloating and flatulence" and "catarrh of the upper respiratory tract".

In folk medicine anise is used for upset stomach, "runny nose" and as an expectorant to increase productive cough, as a diuretic to increase urine flow and as an appetite stimulant. Women use anise to increase milk flow when nursing, start menstruation, treat menstrual discomfort or pain, ease childbirth and increase sex drive. Men use anise to treat symptoms of "male menopause." Other uses include treatment of seizures, nicotine dependence, trouble sleeping (insomnia), asthma and constipation. Anise may be applied directly to the skin to treat lice, scabies and psoriasis.

On the basis of long-standing use and experience, the Committee on Herbal Medicinal Products of EMA recommends the following traditional use indications for aniseed and anise oil: "Traditional herbal medicinal product: For symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating and flatulence; as an expectorant in cough associated with cold."

The above recommended indications are exclusively based upon long-standing traditional use of aniseed. They are supported mainly by experimental data and by experts' opinion, while no data from clinical trials are available.

PHARMACOLOGICAL ACTIVITIES OF ANETHOLE

Antioxidant activity

Fennel oil demonstrated antioxidant capacities as evaluated by two lipid model systems: a modified thiobarbituric acid reactive species assay and a spectrophotometric detection of hydroperoxydienes from linoleic acid in a micellar system, comparable to that of the reference antioxidants *α-tocopherol and butilated hydroxytoluene*. Water and ethanol extracts of fennel seeds showed 99.1 and 77.5% inhibition of peroxidation in linoleic acid system, greater than the same dose *α-tocopherol (36.1%)* (1).

Anti-microbial, anti-fungal activity, antihelmintic and insecticidal activity

Anethole has potent antimicrobial properties, against bacteria, yeast and fungi. Both star anise essential oil and all isolated compounds exhibit anti-HSV-1 activity by direct inactivation of free virus particles in viral suspension assays. Star anise oil reduced viral infectivity by >99% (2).

An acetone extract of aniseed inhibited the growth of a range of bacteria including Escherichia coli and Staphylococcus aureus and also exhibited antifungal activity against Candida albicans and other organisms (3).

Anise oil (0.2 %) alone showed an in vitro activity against Salmonella enteritidis. Aniseed essential oil inhibited the growth of Escherichia coli (minimal inhibitory concentration (MIC): 0.5%), Staphylococcus aureus (MIC: 0.25%), Salmonella typhimurium (MIC: 2.0%) and Candida albicans (MIC: 0.5%) using the agar dilution method (4).

In vitro, anethole has antihelmintic action on eggs and larvae of the sheep gastrointestinal nematode Haemonchus contortus (5).

The insecticidal action of anethole is greater as a fumigant than as a contact agent. (E)-anethole is highly effective as a fumigant against the cockroach Blattella germanica. Anethole is an effective insect repellent against mosquitos (6).

Secretolytic and expectorant effects

A solution of essential oil in 12% ethanol, administered intra-gastrically to anaesthetized guinea pigs at 50 mg/kg b.w., induced a 3 to 6-fold increase in respiratory tract fluid during the first 2 hours after administration. A similar experiment in anaesthetized rats, orally dosed with the oil at 0.0015 ml/kg, resulted in a 28% increase of respiratory tract fluid. Similar results were also observed in cats. An emulsion of 2 drops of the essential oil, administered intragastrically to cats, caused hypersecretion of mucus in the airways and stimulated ciliary removal of mucus, previously inhibited by opium alkaloids (7). An increase of about 12% in mucociliary transport velocity was observed 90 sec after the application of 200 µl of an aniseed infusion (4.6 g per 100 ml of water) to isolated ciliated epithelium of frog oesophagus (8).

Spasmolytic effect on contracted smooth muscles

Pharmacological data show a significant relaxing effect of aniseed alcoholic extracts and essential oil on tracheal and ileal smooth muscles contracted by several contraction-inducing agents (e.g. metacholine and carbachol) (9).

Antinociceptive and anti-inflammatory activity

In the rabbit conjuctival reflex test, solutions of trans-anethole administered into the conjunctival sac increased concentration-dependently the number of stimuli required to evoke the conjuctival reflex ($p<0.01$); the effect was comparable to that of procaine (10).

The effects of anethole in pain models of inflammatory origin was evaluated. Anethole (62.5, 125, 250, and 500 mg/kg) showed an antinociceptive effect in the writhing model induced by intraperitoneal appication of acetic acid, in the second phase of the formalin-induced paw kicking test, in the test with glutamate injected under the ventral surface of the left hind paw, and pain induced by complete Freund adjuvant (CFA) injected into the plantar surface of the hind paw. In contrast, anethole was not able to increase the latency time on the hot plate and decrease the number of flinches during the initial phase of the formalin test in any of the doses tested. The effect of anethole in pain models may be due to a decrease in the production/release of inflammatory mediators (11).

Preincubation of ML1a cells (*a* Line of Human Myeloblastic Leukemia *Cells) with* anethole for 2 h resulted in decreased responses of these cells to the following treatment with TNF (12).

The effects of trans-anethole on IL-1ß and TNF-α level in a rat model of Eschericia coli lipopolysaccharide-induced periodontitis were investigated in comparison with ketoprofen. Administration of either trans-anethole (10 or 50 mg/Kg, *i.p.*) or ketoprofen (10 mg/Kg, *i.p.*) resulted in a similar suppression of IL-1ß and TNF-α production (13).

Anethole and estragole at a dose of 10 mg/kg were also tested in a model of paw edema induced by modulators or inflammatory mediators that participate in carrageenan-induced edema, such as bradykinin, histamine, serotonin, substance P, sodium nitroprusside. Anethole and estragole exhibited similar percent of inhibition of edema induced these substances: substance P (anethole: 64%, estragole: 67%), bradykinin (anethole: 41%, estragole: 42%), histamine (anethole: 70%, estragole: 2%), TNF-α (anethole: 34%, estragole: 44%). However, the percent inhibitions exhibited by these monoterpenes were differentiated from each other in serotonin-induced edema (anethole: 55%, estragole: 30%). Moreover, only estragole (22%), but not anethole, exhibited an inhibitory effect to the sodium nitroprusside edematogenic response (14).

The anti-inflammatory properties of anethole in animal models of nonimmune acute inflammation such as croton oil-induced ear edema and carrageenan-induced pleurisy were investigated. Oral administration of anethole at a dose of 250 and 500 mg/kg reduced both the volume of pleural exudates and the number of migrated leukocytes. The levels of nitric oxide (NO) and prostaglandins (PGE_2) in the inflammatory exudate were reduced by treatment with

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anethole, but IL-1ß and TNF-α levels were not significantly altered. In ear edema, the oral treatment with anethole inhibited the formation of exudate and the activity of myeloperoxidase. These results suggest that the anethole may be effective in controlling some nonimmune acute inflammation-related disease, probably by an inhibitory action on production and/or release of PGE_2 and NO (15).

Gastroprotective activity

Gastroprotective effects of *F. vulgare* essential oil and anethole at doses 50 and 100 mg/kg were investigated in ulcerogenesis induced by ethanol (90%, 1 ml) in rats. Fennel oil and anethole exhibited a significant gastroprotective activity against the erosive damage induced by ethanol. Their similar antiulcer activity could be attributed to the maintenance of a sufficient blood supply in the gastric mucosa through their antiplatelet and vasorelaxant effects. Indeed, they could prevent the disturbance in the gastric circulation generated by ethanol which caused a local vasocongestion with vascular stasis and mucosal damage associated to overproduction of oxygen-derived free radicals (16). Pretreatment with anethole (30 and 300 mg/kg) significantly increased mucus production by the gastric mucosa in the ethanol-induced ulcer model (17).

Oral treatment with anethole at doses of 30, 100, and 300 mg/kg, caused gastroprotection against ethanol- and indomethacin-induced gastric damage. Anethole did not reduce the lesion index in cold-restraint stress-induced ulcers in rats.

The gastric ulcer protective potential of an aqueous suspension of the seeds of ‚Fennel' *Foeniculum vulgare* (FVS) was evaluated against different acute gastric ulcer models in rats induced by pyloric ligation (Shay), hypothermic restraint stress, indomethacin and necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl). Fennel suspension, 250 and 500 mg/kg administered orally (intraperitoneally in Shay rat model) showed dose-dependent ulcer protective effects in all the above models. Besides, FVS offered protection against ethanol-induced depletion of gastric mucus; replenished the reduced nonprotein sulfhydryl concentration and modulated malondialdehyde contents in the gastric tissue. Ethanol induced histopathological lesions in the stomach wall characterized by mucosal hemorrhages and edema that was reversed by FVS. The gastro-protective efficacy of the FVS was probably due to its antisecretory and antioxidant nature by which it strengthened mucosal defensive factors (18).

Anti-ulcerogenic and antioxidant effects of aqueous extracts of *Foeniculum vulgare* (FVE) on ethanol-induced gastric lesions in rats were evaluated. FVE was administered by gavage at doses of 75, 150 and 300 mg/kg, and famotidine (20 mg/kg) was used for comparison. Pre-treatment with FVE and famotidine were found to inhibit ethanol-induced gastric mucosal injury. This protective effect of FVE was highest and statistically significant in the 300 mg/kg group and higher than that in famotidine group. FVE showed an obvious gastroprotective effect and antioxidant properties (19).

Estrogenic effects

Trans-anethole administered orally to immature female rats at 80 mg/kg for 3 days significantly increased uterine weight, to 2 g/kg compared to 0.5 g/kg in controls and 3 g/kg in animals given estradiol valerate subcutaneously at 0.1 µg/rat/day ($p<0.001$). These results confirmed the estrogenic activity of trans-anethole. Other experiments showed that it had no anti-estrogenic, progestational, anti-progestational, androgenic or anti-androgenic activity (20).

Estrogenic activity of trans-anethole at high concentrations has been determined by a sensitive and specific bioassay using recombinant yeast cells expressing the human estrogen receptor. Estrogenic activity described for trans-anethole is not confirmed for aniseed alcoholic extracts on the basis of epidemiological data related to the common use of aniseed alcoholic beverages.

Reproductive toxicity

Trans-anethole exerted a dose-dependent, anti-implantation activity after oral administration to adult female rats on days 1-10 of pregnancy. When compared with control animals (all of which delivered normal offspring on completion of term), trans-anethole administered at 50, 70 and 80 mg/kg inhibited implantation by 33%, 66% and 100% respectively. Further experiments were conducted with the 80 mg/kg dose at different stages of pregnancy. When rats were administered trans-anethole on days 1-2 of pregnancy, normal implantation and delivery occurred; however rats administered anethole on days

3-5 of pregnancy, implantation was completely inhibited; and in those given trans-anethole on days 6-10 of pregnancy three out of five rats failed to deliver at term. No gross malformations of offspring were observed in any of the groups. The results demonstrated that trans-anethole has antifertility activity. From comparison with the days 1-2 group (lack of antizygotic activity), the lower level of delivery in the days 6-10 group was interpreted as a sign of early abortifacient activity (21).

Sedative activity

The pentobarbital-induced sleeping time of mice was increased by 93.5% after simultaneous intra-peritoneal administration of essential oil at 50 mg/kg; trans-anethole gave similar results (22).

SAFETY AND TOXICITY OF ANETHOLE

Anethole is associated with a slight increase in liver cancer in rats, although the evidence is scant and generally regarded as evidence that anethole is not a carcinogen. An evaluation of anethole by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) found its notable pharmacologic properties to be reduction in motor activity, lowering of body temperature, and hypnotic, analgesic, and anticonvulsant effects. At this time, the JECFA summary of these evaluations is that anethole has no safety concern at current levels of intake when used as a flavoring agent. In large quantities, anethole is slightly toxic and may act as an irritant.

The council of Europe (1970) listed trans-anethole giving an acceptable daily intakes of 1.5 mg/kg.

Oral LD_{50} values per kg b.w. were determined for the essential oil as 2.7 g in rats and for trans-anethole as 1.8-5.0 g in mice; 2.1-3.2 g in rats; and 2.16 g in guinea pigs (23).

Mice were fed up to 240 mg trans-anethole/kg/day with the diet for 90 days. Severe loss of body weight and dehydration were reported mainly at doses of greater than or equal to 120 mg/kg/day and were attributed to the poor palatability of the diet and reduced food intake. Males fed orally with doses greater than or equal to 30 mg/kg/day showed increased absolute and relative liver weights, liver glycogen depletion, increased relative thyroid weight. Anethole has a modest enzyme-inducing effect on mouse liver (cytochrome P450 and P448). The increased liver weight was considered to be an adaptive physiological response associated with the enzyme induction properties of trans-anethole, rather than an adverse effect (24).

ANETHOLE OVERDOSE

Ingestion of 1 to 5 ml of anise oil in humans has been associated with nausea, vomiting, seizures and pulmonary edema.

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