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Approach to pharmacological and clinical applications of Anisi aetheroleum

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PEER REVIEW

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Comments

This is a good, interesting and sufficient study about the Anisi aetheroleum which was obtained from *P. anisum*. This article included description of the plant (local names, distribution of the plant all over the world, morphology, medicinal uses). Except for overdose and precaution of the oil used, the author also discussed the preparation of the plant oil, toxicity, pharmacological uses and clinical application in details.

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ABSTRACT

Anisi aetheroleum is the oil obtained from *Pimpinella anisum* L. (*P. anisum*) by steam distillation. *P. anisum* seeds were air-dried, and then the dry seeds were crushed, pulverized, and weighed in sequence for anise oil preparation. *P. anisum* is one of the oldest medicinal plants that belong to family Apiaceae. The fruit of *P. anisum* is harvested in August and September. *P. anisum* is widespread in Asia, Africa and Europe. Local names of *P. anisum* include anise, anisoon, roomy, saunf, sweet cumin and yansoon. The anise oil odour is aromatic while the oil tastes sweet. The average daily dose of Anisi aetheroleum is 0.3 g. *trans*-Anethole is the major ingredient of the anise oil. Anisi aetheroleum also displays a protective action against neurotoxicity. In addition, Anisi aetheroleum increases glucose absorption and reduces urine output in the rat. The plant oil have pharmacological (antimicrobial, hepatoprotective, anticonvulsant, anti-inflammatory, antispasmodic, bronchodilator, estrogenic, expectorant and insecticidal) effects and clinical effects on nausea, constipation, menopausal period, virus, diabetes, obesity and sedative action. Owing to the wide application of Anisi aetheroleum in pharmacological and clinical fields, it is recommended for more clinical trails to discover a new medication from the active constituents of the plant oil in the future to treat human diseases especially chronic ones.

KEYWORDS

Anisi aetheroleum, *Pimpinella anisum*, Apiaceae, Pharmacology, Clinical effect

1. Introduction

Anisi aetheroleum is the oil prepared from *Pimpinella anisum* L. (*P. anisum*). *P. anisum* is one of the oldest medicinal plants that belong to family Apiaceae. It is an annual grassy herb with 30-50 cm high, white flowers, and small green to yellow seeds, which grows in the Mediterranean area, West Asia, Mexico, Egypt, and Europe[1]. The fruit of *P. anisum* is harvested in August and September. Anisi aetheroleum is used as flavouring, digestive, carminative, and for relief of gastrointestinal spasms. Consumption of the plant oil in lactating women increases milk and also relief their infants from gastrointestinal problems[2]. Local names of *P. anisum* include anise, anisoon, roomy, saunf, sweet cumin and yansoon. In the food industry, the anise oil is used as flavoring and aromatic agent for fish products, ice cream, sweets, and gums[1,3]. Anisi aetheroleum is used as analgesic in migraine and also as carminative, aromatic, disinfectant and diuretic in traditional medicine[4]. The anise oil has warm and dry nature. It can increase milk secretion, promote menstruation, facilitate birth, alleviate the symptoms of the male climacteric, and increase libido. It is also effective in polishing of teeth. In some traditional texts, anise oil is mentioned for melancholy, nightmare, and also in treatment of epilepsy and seizure[5,6]. Anisi aetheroleum was analyzed by gas chromatography. Gas chromatography-mass spectra showed the presence of *trans*-

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anethole (93.9%) and estragole (2.4%). Other compounds that were found with concentration higher than 0.06% were (E)-methyleugenol, α-cuparene, α-himachalene, β-bisabolene, *p*-anisaldehyde, and *cis*-anethole[3]. Some other compounds such as *trans*-pseudoisoeugenyl 2-methylbutyrate, *p*-anisaldehyde and methylchavicol were also identified in anise oil[7]. The other constituents of the plant oil, present in amounts of 1%-5% were *cis*-anethole, carvone, β-caryophyllene, dihydrocarvyl acetate, estragole and limonene[8]. Anisi aetheroleum is obtained from *P. anisum* grown in Spain, Germany, Italy and other European countries. The major constituents were anethole (>90%), γ-himachalene (2%-4%), *p*-anisaldehyde (<1%), methylchavicol (0.9%-1.5%), *cis*-pseudoisoeugenyl 2-methylbutyrate (3%), and *trans*-pseudoisoeugenyl 2-methylbutyrate (1.3%)[9]. Neophytadiene was isolated from the anise oil[10]. 4-(β-D-glucopyranosyloxy) benzoic acid, one of the phenolic glycosides, was also isolated from anise oil[11]. Four aromatic compound glucosides, an alkyl glucoside and a glucide were isolated from the anise oil as new compounds. The structures of the new compounds were clarified as (E)-3-hydroxy-anethole β-D-glucopyranoside, (E)-1'-(2-hydroxy-5-methoxyphenyl) propane β-d-glucopyranoside, 3-hydroxyestragole β-d-glucopyranoside, methyl syringate 4-*O*-β-D-glucopyranoside, hexane-1,5-diol 1-*O*-β-D-glucopyranoside and 1-deoxy-l-erythritol 3-*O*-β-dglucopyranoside[12]. Isolation and structure elucidation of flavonoid constituents from the anise oil by means of chromatography on cellulose columns lead to isolation of quercetin 3-glucuronide, rutin, luteolin 7-glucoside, isoorientin, and isovitexin as crystalline compounds, and apigenin 7-glucoside and a luteolin glycoside as noncrystalline compounds[13]. A silver ion high performance liquid chromatography procedure was used to determine the fatty acids composition of Anisi aetheroleum, and showed the positionally isomeric 18:1 fatty acids oleic acid (*cis* 9-18:1), petroselinic acid (*cis* 6-18:1), and *cis*-vaccenic acid (*cis* 11-18:1), in the plant oil by a single gradient run on a single cation exchange column laboratory converted to the silver ion form[14]. Also three lignin-carbohydrate-protein complexes were isolated from a hot water extract of the seeds of *P. anisum* by combination of anion exchange, gel filtration, and hydrophobic interaction column chromatography[15].

This review should act to stimulate a thought process on importance of the pharmacological and clinical applications of the Anisi aetheroleum and the usefulness of this plant oil in pharmaceutical industry process.

2. Preparation of Anisi aetheroleum

P. anisum seeds were air-dried in an oven at 40 ℃ for 4 d and then the dry seeds were crushed, pulverized and weighed in sequence for oil preparation. Distilled water was placed in the steam generator, and then the steam generator started heating to produce steam. The seed powder was placed in the round bottom flask. A vigorous current of steam from steam generator passed through the round bottom flask. A part of the steam condensed in the round bottom flask. As more and more steam passed, the steam volatile components of the seed powder passed through the condenser along with steam. These contents on condensation were collected in the receiver. The contents in the round bottom flask were heated by a Bunsen burner to prevent excessive condensation of steam. The process of steam distillation was continued for about half an hour. The distillate was transferred to a separating funnel and extracted with petroleum ether 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 was left behind in the distillation flask.

3. Toxicology of Anisi aetheroleum

El-Wahab and Moram investigated the effect of *trans*-anethole of Anisi aetheroleum[16]. The anethole induced a significant decrease in body weight, hemoglobin concentration and red blood cell count. Also there was a significant decrease in reduced glutathione content, glutathione-S-transferase (GST) and superoxide dismutase activities in both blood and liver of rats in test groups compared to control group. On the other hand, a significant increase in serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase activities, bilirubin, urea, creatinine, total protein and albumin were observed in all test groups when compared with control group. In a study, combinations of two cytotoxic phytochemicals (anethole and curcumin) from Anisi aetheroleum were applied in binary combination with platinum drugs (cisplatin and oxaliplatin) against three epithelial ovarian cancer cell lines: parental A2780, $A2780^{cisR}$ (cisplatin-resistant) and $A2780^{ZD0473R}$ (ZD0473-resistant). Cell viability was quantified using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide reduction assay and the combined drug action was analyzed. Greatest synergism was observed when the phytochemical Anisi aetheroleum anethole was added first followed by the platinum drug 2 h later, and additiveness to antagonism in combined drug action was observed when the two compounds were administered as a bolus[17]. The essential oils from four Apiaceae species including anise oil were evaluated for their antifeedant, growth inhibitory, and insecticidal activities against *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) fourth instar larvae. Essential oils were characterized by gas chromatography and mass spectrometry. The results showed that anti-insect activity the essential oils varied according to plant specie/composition, type, and exposure period. The plant oils containing *trans*-anethole exerted acute effects on larvae feeding and growth. For the most active essential oils/compounds, dose-dependent toxicity was determined and inverse relationships of LC_{50} with time were established[18]. Natsch and Haupt examined the suspected prohaptens such as *trans*-anethole as well as methyl isoeugenol and eugenol as skin-sensitizing prohaptens in a modified KeratinoSens assay. The result obtained showed no, or only weak, gene induction in absence of S9 fractions, and a significantly enhanced luciferase induction in presence of S9, proving their prohapten status[19]. On another study, Chang *et al*. studied the toxicity of 12 insecticides and 3 essential oils which contained anethole as a major constituent[20]. Based on the results, mixture of *Bacillus thuringiensis* var. *israelensis* and anethole were significantly more toxic against *Aedes albopictus* larvae (0.023 7 mg/L) and *Anopheles sinensis* larvae (0.054 1 mg/L) than either *Bacillus thuringiensis* var. *israelensis* (1.788 4 and 2.168 1 mg/L) or anethole (16.660 0 and 25.110 0 mg/L) alone.

4. Pharmacological effects

4.1. Antimicrobial effect

Gülçin *et al*. examined the antimicrobial effect of Anisi aetheroleum against 10 bacterial species and *Candida albicans* (*C. albicans*) with disc diffusion method[21]. In this study, the plant oil showed significant inhibitory activity against all tested bacteria. However, the antimicrobial effect of Anisi aetheroleum was effective against *C. albicans*. Anisi aetheroleum also showed antibacterial activity against *Micrococcus luteus* and *Mycobacterium smegmatis*[22]. Synergic antibacterial activity of *Thymus vulgaris* and *P. anisum* essential oils was evaluated against 9 pathogenic bacteria. Essential oils of these plants exhibited antibacterial activity against most tested pathogens, and the maximum effect was

observed against *Staphylococcus aureus*, *Bacillus cereus*, and *Proteus vulgaris*. However, combination of essential oils of these plants showed an additive effect against most tested pathogens especially *Pseudomonas aeruginosa*[23]. In addition to antibacterial activity, Anisi aetheroleum showed significant inhibitory activity against fungi, and the most active component was anethol[24]. Anisi aetheroleum exhibitd stronger antifungal activity against yeasts and dermatophytes. The maximum inhibition was found in *Candida parapsilosis*, followed by *C. albicans*, *Candida glabrata*, and *Geotrichum* spp[25]. Antifungal activity of Anisi aetheroleum was also reported against *Alternaria alternata*, *Aspergillus niger* and *Aspergillus parasiticus*[3,25]. Anisi aetheroleum showed total fungal inhibition at 1 500 μL/L. The plant oil affected the ochratoxin A biosynthesis pathway of both *Aspergillus* species, so Anisi aetheroleum was used as an effective non-toxic biopreservative against ochratoxin A contamination in stored peanuts[26]. The antimicrobial activity of 15 essential oils was investigated against *Clostridium butyricum, Clostridium hystoliticum, Clostridium intestinale, Clostridium perfringens* and *Clostridium ramosum*, and Anisi aetheroleum showed the highest antimicrobial activity against *Clostridium butyricum*[27].

4.2. Hepatoprotective effect

Since Anisi aetheroleum can protect the protein metabolism cycle in the liver, it can protect the liver against the hepatotoxic materials[28,29]. The anise oil showed hepatoprotective activity through *in vitro* and *in vivo* antioxidant potential; the ethanolic extract of aniseed displayed scavenging activity against nitric oxide (NO), superoxide and 1,1-diphenyl, 2-picryl hydrazyl radicals and reducing power in a concentration-dependent manner[30]. Anisi aetheroleum exerted hepatoprotective effect through antioxidant activity by serum antioxidant enzymes as well as oxidative stress and peroxides inhibition[31].

4.3. Anticonvulsant effect

The anticonvulsant effect of Anisi aetheroleum was studied against seizures induced by pentylenetetrazole (PTZ) or maximal electroshock in male mice. The study revealed that the plant oil increased the threshold of clonic seizures induced by *i.v.* injection of PTZ, and it can also block tonic convulsions induced by *i.p.* injection of PTZ. Moreover, the plant oil possesses anticonvulsant activity against tonic seizures induced by maximal electroshock[32]. The effect of Anisi aetheroleum on picrotoxin-induced seizure in mice was studied by Heidari and Ayeli, and the results showed that Anisi aetheroleum caused an increased delay at the onset of seizure in the mice which had been pretreated with different doses of the plant oil, and the most effective dose was 200 mg/kg. In addition, this dose delayed the time of death in mice more satisfactory than phenobarbital (40 mg/kg) in delaying death time[32]. The anise oil produced hyperexcitability through enhancement of Ca^{2+} channels activity or inhibition of voltage and/or Ca^{2+} dependent K^{+} channels activity underlying post-hyperpolarization potential[33,34].

4.4. Anti-inflammatory effect

Anethole orally administered in a subacute treatment to mice (30 mg/kg/day for 5 d) showed significant antithrombotic activity, preventing the paralysis induced by intravenous injection of collagen-epinephrine (83% protection). At the antithrombotic dosage the anethole were free from prohemorrhagic side effect at variance with acetylsalicylic acid used as reference drug. Furthermore, the anethole (100 mg/kg oral administration) provided significant protection from ethanol induced gastric lesions in rats[35]. Anethole (10 μmol/L) inhibited platelet aggregation and the formation of thromboxane B2 in plasma in response to adenosine diphosphate, epinephrine and arachidonic acid. Anethole inhibits platelet aggregation by inhibiting thromboxane synthesis and preventing arachidonic acid release[36]. The anise oil has anti-inflammatory effect as strong as indomethacin's[37]. 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. Levels of NO and prostaglandins in the inflammatory exudate were reduced by treatment with anethole, but levels of tumor necrosis factor-α and interleukin-1β were not significantly altered. In ear edema, the oral treatment with anethole inhibited the formation of exudate and the activity of myeloperoxidase, but not after topical administration. These results suggest that the anethole is effective in controlling some nonimmune acute inflammation-related disease, by an inhibitory effect on production and/or release of prostaglandins and NO[38].

4.5. Relaxant effect

The anise oil showed significant relaxant effects. The results of a study showed parallel rightward shifts of methacholine-response curves and significant increase in EC_{50} with the presence of anise oil. The results also showed that the relaxant effect of anise oil is not due to an inhibitory effect of histamine or stimulatory effect of beta(2)-adrenergic receptors, but due to inhibitory effects on muscarinic receptors[39]. The anethole of the plant oil improved compliance in women taking oxybutinin hydrochloride for detrusor instability[40]. In another study, the effect of chronic treatment with anethole on the phosphatidylinositol (PI) turnover and cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) accumulation in rat submaxillary glands has been compared with the effect of chronic treatment with atropine and a cholinesterase inhibitor, diisopropylfluorophosphate. Experiments were performed 24, 48 and 24 h after the last dose of anethole, atropine and dyflos, respectively. Anethole enhanced carbachol-stimulated [32P] incorporation into phosphatidic acid in the submaxillary glands slices, while dyflos showed no effect. Pilocarpine-stimulated *in vivo* incorporation of [3H] myoinositol into inositol phosphates was significantly enhanced by anethole, but not by atropine or dyflos. Phospholipase C-dependent hydrolysis of PI 4,5-bisphosphate was significantly enhanced by anethole, but not by dyflos. Pilocarpine-stimulated *in vivo* accumulation of cAMP and cGMP was enhanced by anethole, but dyflos reduced cAMP accumulation without affecting cGMP accumulation. The enhancement of PI turnover and cyclic nucleotide accumulation seems to contribute to the development of supersensitivity of the salivary gland caused by chronic treatment with anethole, while reduction of cAMP accumulation may be responsible for the sub-sensitivity caused by dyflos[41]. The plant oil was successful for plasma to serve as alternative organic phase in hollow fibre based liquid-phase microextraction, eliminating the use of hazardous organic solvents[42].

4.6. Bronchodilatory effect

Anisi aetheroleum had bronchodilatory effects[39]. These effects were related to anethole consitutent of the plant oil where anethole (250 mg/kg) decreased total protein concentrations, numbers of inflammatory

cells, including neutrophils and macrophages, and the inflammatory mediators matrix metalloproteinase 9, tumor necrosis factor-alpha, interleukin-6, NO in bronchoalveolar lavage fluid. In addition, pretreatment with anethole decreased lipopolysaccharide-induced histopathological changes[43]. Also, anethole inhibited carrageenan-induced edema at doses of 3, 10 and 30 mg/kg at 60 to 240 min after induction. Anethole inhibited edema induced by substance P, bradykinin, histamine and tumor necrosis factor-alpha[44]. The *trans*-anethole of Anisi aetheroleum effectively inhibited lipopolysaccharide-induced NO production, and it was the most abundant plant oil constituents, with IC_{50} value of 102.7 μg/mL[45]. A finding of a study described that only the plant extract contained 60% ethanol showed concentration dependently relaxed acetylcholine-pre-contracted tissues, but two other hydro alcoholic extracts cannot produce relaxation. Study of the possible mechanisms underlying the relaxant effect showed that this effect is mainly dependent on the activation of the NO-cGMP pathway[46].

4.7. Estrogenic effect

Anisi aetheroleum have been used as estrogenic agents for millennia. The plant oil increases milk secretion, promotes menstruation, facilitates birth, alleviates the symptoms of the male climacteric, and increases libido. The plant is used in the development of synthetic estrogens where the main constituent of the plant oil anethole, has been considered as the active estrogenic agent[47]. Anethole causes premature thelarche, which is a common disorder characterized by breast development, usually younger than 2 years, with no other signs of puberty. It is usually associated with adrenal or ovarian disorders, hypothyroidism, and use of exogenous hormones or drugs, and it may also be associated with long-term use of herbal medicine[48]. Anethole from the plant oil has been used for alleviation of menopausal symptoms, prevention of osteoporosis, heart disease and cancer[49].

4.8. Expectorant effect

The anise oil enhanced significantly glucose absorption in the rat jejunum and increased the Na^+/K^+-ATPase activity in a jejunal homogenate in a dose dependent manner. However, the oil exerted no effect on water absorption in the colon and did not alter the activity of the colonic Na^+/K^+-ATPase. When added to drinking water, it reduced the volume of urine produced in the rat and increased the activity of the renal Na^+/K^+-ATPase even at extremely low concentrations. It was concluded that anise oil increases glucose absorption by increasing the activity of the Na^+/K^+-ATPase and consequently the sodium gradient needed for the sugar transport. Its anti-diuretic effect is also mediated through a similar mechanism in the kidney whereby a stimulation of the Na^+-K^+ pump increases tubular sodium reabsorption and osmotic water movement[50]. This effect was related to *trans*-anethole of the oil, and the administration of 200 mg/L anethole significantly inhibited the incidence and multiplicity of both invasive and non-invasive adenocarcinomas; whereas feeding of 100 mg/L anethole suppressed only invasive adenocarcinomas of the colon. GST, NAD-(P)H-dependent quinone reductase, and UDP-glucuronosyltransferase activities in colonic mucosa and tumor and liver were significantly elevated in animals fed on anethole compared to those fed on the control diet. Anethole inhibited azoxymethane-induced colon carcinogenesis with increased activities of phase II enzymes such as GST, NAD(P)H-dependent quinone reductase, and UDP-glucuronosyl transferase in the liver and colon[51,52].

4.9. Insecticidal effect

Anisi aetheroleum was tested for the insecticidal activity against larvae of *Lycoriella ingenua* using a fumigation bioassay. The anise oil showed good insecticidal activity against the larvae. *trans*-Anethole was the second most toxic against larvae of *Lycoriella ingenua*[53,54]. Anisi aetheroleum was highly effective as larvicidal and ovicidal against three mosquito species[55]. Also the anise oil showed repellency against *Culex pipiens*[56]. In another study, the exposure to vapour from anise oil resulted in 100% mortality of the eggs of two stored-product insects (the confused flour beetle, *Tribolium confusum*, and Mediterranean flour moth, *Ephestia kuehniella*)[57].

5. Clinical effect

5.1. Effect on nausea

The treatment contains Anisi aetheroleum and oils from *Foeniculum vulgare* var. *dulce, Anthemis nobilis*, and *Mentha piperita,* which was examined in twenty-five patients who were suffering from the symptoms of nausea in a hospice and palliative care program. The symptoms of majority of patients who used this treatment were significantly relieved. However, all patients in this study were also using a variety of other treatments for their symptoms[58].

5.2. Effect on constipation

Anisi aetheroleum and the essential oils were prepared from *Foeniculum vulgare* Miller, *Sambucus nigra* L. and *Cassia angustifolia,* which were evaluated in trial including 20 patients with chronic constipation. The primary endpoint was colonic transit time, measured radiologically. The secondary endpoints included number of evacuations per day, perception of bowel function, adverse effects and quality of life. Anisi aetheroleum revealed significant laxative effect when compared with placebo. This effect was demonstrated by a decrease in colonic transit time as well as an increase in the number of daily evacuations. Although quality of life did not show significant differences during the study periods and no significant differences were observed in terms of adverse effects throughout the study period, Anisi aetheroleum oil can be a safe alternative option for the treatment of constipation[59].

5.3. Effect on menopause

The effect of anise oil on menopause was investigated in 72 postmenopausal women. In this clinical study, consumption of 3 capsules of anise oil (each capsule contains 100 mg of Anisi aetheroleum) for 4 weeks leads to significant reduction in a period, frequency and intensity of hot flush in menopausal and postmenopausal women[60].

5.4. Effect on dysmenorrhea

In a clinical study, the effect of capsule containing anise oil, celery and saffron was compared with that of mefenamic acid capsule in 180 female students (with age of 17-28 years) with primary dysmenorrhea. The results showed significant reduction in pain intensity in both herbal and mephnamic acid group compare to placebo group. Also the results revealed that the effectiveness of herbal capsule was better than mephnamic acid in pain relief and can be a suitable alternative in

primary dysmenorrhea[33].

5.5. Effect on virus

Anisi aetheroleum is capable of exerting a direct effect on herpes simplex virus type 1 and 2, human cytomegalovirus and measles virus strains. The effects of the compounds of Anisi aetheroleum on messenger RNA (mRNA) and protein expression of inducible nitric oxide synthase (iNOS) in RAW 264.7 cells showed that they induced mRNA iNOS expression in a time-dependent manner. Furthermore, they induced expression of both interleukin-1β and interleukin-10 mRNAs. Also the effects of anise oil in activation of macrophage were investigated after murine macrophage cells had been incubated with these compounds for 20 h, and the NO production was enhanced in dose-dependent manner. The effect of the plant oil on mRNA and protein expression of iNOS in cells showed that they induced mRNA iNOS expression in a time-dependent manner. Furthermore, they also induced expression of both interleukin-1β and interleukin-10 mRNAs. These results suggest that the plant oil complexes possessed potency as functional food ingredients against infectious diseases and were useful in the treatment of infection caused by drug-resistant viruses[15,61].

5.6. Effect on diabetes and obesity

Anisi aetheroleum (5 g/day) was administered to two groups of type 2 diabetes patients for 60 d. The results indicated 11% rise of fasting blood glucose in control and 36% decrease in anise oil-treated type 2 diabetics. Also significant decrease in serum cholesterol and triglycerides by plant oil which was used to treat patient was observed. Protein oxidation in serum and lipid peroxidation in erythrocytes and plasma was decreased in both treated groups as compared with the initial values. The oil treated group showed rise in serum β-carotene and vitamin A levels which could have resulted in a significant decrease in lipid peroxidation in red blood cells and plasma, and also rise in vitamin C was detected[31].

5.7. Sedative effect

A clinical study including 80 patients of the anise oil showed significant sedative effect similar to morphine and aspirin did[62]. The plant oil treatment restored the motor impairment caused by midazolam drug. The pretreatment with the oil caused significant shortage of pentobarbital induced sleeping time when compared to control. The decrease in antidepressant effect of imipramine and fluoxetine drugs was diminished by the pretreatment with Anisi aetheroleum[63].

6. Overdose side effect

Anethole of the anise oil enhances the absorption of etodolac significantly. This is consistent with the fact that hydrophobic plant oil is effective in the percutaneous absorption of lipophilic drugs. The etodolac creates gastrointestinal disturbances[64]. Anethole at a dose of 75 mg had a severe side effects such as abdominal discomfort and flatulence, which were seen in 7 patients (44%) from 16 patients. One of these patients in addition had diarrhea and nausea. The side effects were persistent during treatment only[65]. Over dose range of anise oil, the elimination of ^{14}C-labeled urinary metabolites increased significantly in both rats and mice. There was a species-specific regioselectivity in the side-chain oxidation of anethole. The rat favored the epoxidation route, resulting in the elimination in the urine of two 1-(4'-methoxyphenyl)propane-1,2-diol isomers, elimination of which together rose from 2%-15% of the dose over the dose range studied[66].

7. Precautions

Anise oil anethole is non-hepatocarcinogenic alkenylbenzenes which was studied in a carcinogenicity bioassay, but the dose can convert anethole from non-hepatocarcinogenic to hepatocarcinogenic. So anethole would be weakly hepatocarcinogenic if studied at a dose level of 2 mmol/kg body weight/day for 2 years in male F344 rats. Therefore suggesting that these chemicals should be a higher priority relative to other untested alkenylbenzenes for evaluation in the carcinogenicity bioassay[67]. Oral administration of anise oil anethole also exerted a pharmacodynamic action in some brain regions of the mice, and proteasome peptidase activities were significantly elevated in the cerebral cortex-hippocampus. Moreover, tissue from anethole-treated mice and cell lysates obtained from anethole-incubated murine neuroblastoma cells exhibited the enhanced capacity to degrade mutant human SOD1G93A protein. These results indicate that the catalytic subunits of the 26S proteasome are inducible in multiple tissues of mouse including brain by exogenous chemical treatment. Increased proteasome expression by inducers may have a role in protection/attenuation of protein aggregate-mediated disorders[68]. The oral treatment with anethole (30-300 mg/kg) caused dose-dependent gastroprotection against ethanol- and indomethacin-induced gastric damage, but did not change cold-restraint stress-induced ulcers in rats. Furthermore, anethole (30-300 mg/kg) significantly increased the mucus production by the gastric mucosa, measured by Alcian blue binding, in ethanol-induced ulcer model. However, anethole did not promote significant alteration in gastric production of non-protein sulfhydryl groups. In pylorus-ligated model, anethole (30-300 mg/kg) had a significant effect on the volume of gastric juice, pH, or total acidity. The anise oil anethole can not only reduce the risk of acquiring specific cancer but also have been shown to suppress cancer cell proliferation, inhibit growth factor signaling pathways, induce apoptosis, inhibit nuclear factor-κB, AP-1, Akt, MAPK, Wnt, Notch, p53, AR, ER, and JAK-STAT, *etc.*, activation pathways, inhibit angiogenesis, suppress the expression of antiapoptotic proteins, and inhibit cyclooxygenase-2[69]. In another study, Yang *et al.* examined the antimicrobial mechanism of *Illicium verum* with killing curves and scanning electron microscopy observation, and revealed the antibacterial activity with a minimum inhibitory concentration value of 0.15-0.70 mg/mL and 0.11 mg/mL respectively[70].

8. Conclusion

Anisi aetheroleum is the oil obtained from *P. anisum* by steam distillation. *P. anisum* is one of the oldest medicinal plants that belong to family Apiaceae. Anisi aetheroleum constituents include the presence of *trans*-anethole (93.9%) and estragole (2.4%), and other compounds that were found with concentration higher than 0.06% were (E)-methyleugenol, α-cuparene, α-himachalene, β-bisabolene, *p*-anisaldehyde, and *cis*-anethole. Anisi aetheroleum is used as flavouring, digestive, carminative, and for relief of gastrointestinal spasms. The average daily dose of Anisi aetheroleum is 0.3 g. The plant oil have pharmacological (antimicrobial, hepatoprotective, anticonvulsant, anti-inflammatory, antispasmodic, bronchodilator, estrogenic, expectorant and insecticidal) effects and clinical effect on nausea, constipation, menopausal period, virus,

diabetes, obesity and sedative action.

Conflict of interest statement

I declare that I have no conflict of interest.

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Comments

Background

Anisi aetheroleum is the essential oil obtained by steam distillation from *P. anisum* (family: Apiaceae). It is indigenous to the Eastern Asia, Europe and Mediterranean region. The major constituents are *trans*-anethole, *cis*-anethole, methylchavicol, linalool and *p*-anisaldehyde. Anisi aetheroleum is a clear, colourless or pale yellow liquid. The plant is used for the treatment of dyspepsia, inflammation of the respiratory tract and chronic bronchitis. Also it is used as an aphrodisiac, carminative, emmenagogue, galactagogue and insecticide. Except for its clinical application, this plant oil has antimicrobial, hepatoprotective, anticonvulsant, anti-inflammatory, antispasmodic, bronchodilator, and expectorant effects.

Research frontiers

This article described preparation, toxicology, pharmacological and clinical effects of Anisi aetheroleum. In addition, overdose side effect and precaution were discussed. Futhermore, the botanical features and medicinal application of the plant oil were also included. This article can be a guide to the future pharmacological and clinical applications of the plant oil to develop new and effective plant constituents which will be used in the pharmaceutical companies.

Related reports

The manuscript contains preparation of the oil, toxicology, morphological features of the medicinal plant, diversity and widespread of the plant, different local names, pharmacological uses and clinical application of the plants oil. The daily dose of the oil, overdose and precaution of the plant oil were also discussed.

Innovations and breakthroughs

This article explores the preparation of Anisi aetheroleum obtained from *P. anisum* and the diversity of this plant in many regions of the world. It contains morphological describe of the plant as well as medicinal uses. The author explainsed in details pharmacological and clinical effects of the plant oil. He also focused on the overdose and precautions of the oil applications in this paper. This review is a good and attractive study with the pharmacological and clinical importance of the plant oil.

Applications

From the data collected, it has been clear that the plant oil has many effects like antimicrobial, hepatoprotective, anticonvulsant, anti-inflammatory, antispasmodic, bronchodilator, estrogenic, expectorant activities and clinical application. The side effect and precautions of the oil used were also explained in the review. This good and scientific study supports and explores the importance of pharmacological activities and clinical application of the plant oil.

Peer review

This is a good, interesting and sufficient study about the Anisi aetheroleum which was obtained from *P. anisum*. This article included description of the plant (local names, distribution of the plant all over the world, morphology, medicinal uses). Except for overdose and precaution of the oil used, the author also discussed the preparation of the plant oil, toxicity, pharmacological uses and clinical application in details.

References

[1] Salehi Surmaghi MH. *Medicinal plants and phytotherapy*. Tehran: Donyay Taghziah Press; 2010, p. 58-69.

[2] Zargari A. *Medicinal plants.* Tehran: Tehran University Press; 1996, p. 112-120.

[3] Özcan MM, Chalchat JC. Chemical composition and antifungal effect of anise (*Pimpinella anisum* L.) fruit oil at ripening stage. *Ann Microbiol* 2006; **56**(4): 353-358.

[4] Amin GR. *Poular medicinal plants of Iran*. Tehran: Tehran University of Medical Science Press; 2005, p. 21-38.

[5] Mirheydar H. *Herbal information: usage of plants in prevention and treatment of diseases*. Tehran: Islamic Culture Press Center; 2001, p. 72-81.

[6] Aghili Khorasani MH. [*Store of medicine*]. Qom: Bavardaran Press; 2001, p. 24-39. Persion.

[7] Orav A, Raal A, Arak E. Essential oil composition of *Pimpinella anisum* L. fruits from various European countries. *Nat Prod Res* 2008; **22**(3): 227-232.

[8] Embong MB, Hadziyev D, Molnar S. Essential oils from spices grown in Alberta. Anise oil (*Pimpinella anisum*). *Can J Plant Sci* 1997; **57**: 681-688.

[9] Rodrigues VM, Rosa PT, Marques MO, Petenate AJ, Meireles MA. Supercritical extraction of essential oil from aniseed (*Pimpinella anisum* L.) using CO_2: solubility, kinetics, and composition data. *J Agric Food Chem* 2003; **51**(6): 1518-1523.

[10] Burkhardt G, Reichling J, Martin R. Becker H. Terpene hydrocarbons in *Pimpinella anisum* L. *Pharm Weekbl Sci* 1986; **8**(3): 190-193.

[11] Dirks U, Herrmann K. 4-(β-D-glucopyranosyloxy) benzoic acid, a characteristic phenolic constituent of the Apiaceae. *Phytochemistry* 1984; **23**(8): 1811-1812.

[12] Fujimatu E, Ishikawa T, Kitajima J. Aromatic compound glucosides, alkyl glucoside and glucide from the fruit of anise. *Phytochemistry* 2003; **63**(5): 609-616.

[13] Kunzemann J, Herrmann K. [Isolation and identification of flavon(ol)-O-glycosides in caraway (*Carum carvi* L.), fennel (*Foeniculum vulgare* Mill.), anise (*Pimpinella anisum* L.), and coriander (*Coriandrum sativum* L.), and of flavon-C-glycosides in anise. I. Phenolics of spices]. *Z Lebensm Unters Forsch* 1977; **164**(3): 194-200. German.

[14] Denev RV, Kuzmanova IS, Momchilova SM, Nikolova-Damyanova BM. Resolution and quantification of isomeric fatty acids by silver ion HPLC: fatty acid composition of aniseed oil (*Pimpinella anisum*, Apiaceae). *J AOAC Int* 2011; **94**(1): 4-8.

[15] Lee JB, Yamagishi C, Hayashi K, Hayashi T. Antiviral and immunostimulating effects of lignin-carbohydrate-protein complexes from *Pimpinella anisum*. *Biosci Biotechnol Biochem* 2011; **75**(3): 459-465.

[16] El-Wahab HM, Moram GS. Toxic effects of some synthetic food colorants and/or flavor additives on male rats. *Toxicol Ind Health* 2013; **29**(2): 224-232.

[17] Nessa MU, Beale P, Chan C, Yu JQ, Huq F. Studies on combination of platinum drugs cisplatin and oxaliplatin with phytochemicals anethole and curcumin in ovarian tumour models. *Anticancer Res* 2012; **32**(11): 4843-4850.

[18] Sousa RM, Rosa JS, Oliveira L, Cunha A, Fernandes-Ferreira M. Activities of Apiaceae essential oils against armyworm, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). *J Agric Food Chem* 2013; **61**(32): 7661-7672.

[19] Natsch A, Haupt T. Utility of rat liver S9 fractions to study skin-sensitizing prohaptens in a modified KeratinoSens assay. *Toxicol Sci* 2013; **135**(2): 356-368.

[20] Chang KS, Shin EH, Yoo DH, Ahn YJ. Enhanced toxicity of binary mixtures of *Bacillus thuringiensis* subsp. *israelensis* and three essential oil major constituents to wild *Anopheles sinensis* (Diptera: Culicidae) and *Aedes albopictu*s (Diptera: Culicidae). *J Med Entomol* 2014; **51**(4): 804-810.

[21] Gülçin İ, Oktay M, Kıreççı E, Küfrevioğlu Öİ. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chem* 2003; **83**(3): 371-382.

[22] Ates DA, Erdogrul OT. Antimicrobial activities of various medicinal and commercial plant extracts. *Turk J Biol* 2003; **27**: 157-162.

[23] Al-Bayati FA. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. *J Ethnopharmacol* 2008; **116**(3): 403-406.

[24] Shukla HS, Tripathi SC. Antifungal substance in the essential oil of anise (*Pimpinella anisum* L.). *Agric Biol Chem* 1987; **51**(7): 1991-1993.

[25] Kosalec I, Pepeljnjak S, Kuatrak D. Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., Apiaceae). *Acta Pharm* 2005; **55**(4): 377-385.

[26] Passone MA, Girardi NS, Etcheverry M. Evaluation of the control ability of five essential oils against *Aspergillus section* Nigri growth and ochratoxin A accumulation in peanut meal extract agar conditioned at different water activities levels. *Int J Food Microbiol* 2012; **159**(3): 198-206.

[27] Kačániová M, Vukovič N, Horská E, Salamon I, Bobková A, Hleba L, et al. Antibacterial activity against *Clostridium* genus and antiradical activity of the essential oils from different origin. *J Environ Sci Health B* 2014; **49**(7): 505-512.

[28] Shah B, Seth A. *Textbook of pharmacognosy and phytochemistry*. 4th ed. Amsterdam: Elsevier Health Sciences; 2012, p. 84-93.

[29] *European pharmacopoeia*. 8th ed. Strasbourg: The European Pharmacopoeia Commission; 2013, p. 216-229.

[30] Rajeshwari CU, Abirami M, Andallu B. *In vitro* and *in vivo* antioxidant potential of aniseeds (*Pimpinella anisum*). *Asian J Exp Biol Sci* 2011; **2**(1): 80-89.

[31] Rajeshwari U, Shobha I, Andallu B. Comparison of aniseeds and coriander seeds for antidiabetic, hypolipidemic and antioxidant activities. *Spatula DD* 2011; **1**(1): 9-16.

[32] Heidari MH, Ayeli M. [Effects of methyl alcoholic extract of *Pimpinella anisum* on picrotoxin induced seizure in mice and its probable mechanism]. *Sci J Kurd Univ Med Sci* 2005; **10**(3): 1-8. Persian.

[33] Karami NK, Moattar F, Ghahiri A. Comparison of effectiveness of an herbal drug (celery, saffron, Anise) and mephnamic acid capsule on primary dismenorrhea. *J Gonabad Univ Med Sci* 2008; **14**(1): 11-19.

[34] Janahmadi M, Farajnia S, Vatanparast J, Abbasipour H, Kamalinejad M. The fruit essential oil of *Pimpinella anisum* L. (Umblliferae) induces neuronal hyperexcitability in snail partly through attenuation of after- hyperpolarization. *J Ethnopharmacol* 2008; **120**(3): 360-365.

[35] Tognolini M, Ballabeni V, Bertoni S, Bruni R, Impicciatore M, Barocelli E. Protective effect of *Foeniculum vulgare* essential oil and anethole in an experimental model of thrombosis. *Pharmacol Res* 2007; **56**(3): 254-260.

[36] Selley ML, McGuiness JA, Bartlett MR, Ardlie NG. Effect of anethole dithiolthione on human platelet aggregation. *Arzneimittelforschung* 1992; **42**(3): 318-321.

[37] Tas A, Özbek H, Atasoy N, Altug ME, Ceylan E. Evaluation of analgesic and antiinflammatory activity of *Pimpinella anisum* fixed oil extract. *Indian Vet J* 2006; **83**(8): 840-843.

[38] Domiciano TP, de Oliveira Dalalio MM, Silva EL, Ritter AMV, Estevão-Silva CF, Ramos FS, et al. Inhibitory effect of anethole in nonimmune acute inflammation. *Naunyn Schmiedebergs Arch Pharmacol* 2013; **386**(4): 331-338.

[39] Boskabady MH, Ramazani-Assari M. Relaxant effect of *Pimpinella anisum* on isolated guinea pig tracheal chains and its possible mechanism(s). *J Ethnopharmacol* 2001; **74**(1): 83-88.

[40] Arango O. The use of salivary stimulant pastilles to improve compliance in women taking oxybutinin hydrochloride for detrusor instability. *Br J Urol* 1998; **81**(4): 652.

[41] Ukai Y, Taniguchi N, Yamazaki A, Kimura K. Enhancement of phosphatidylinositol turnover and cyclic nucleotide accumulation by chronic anethole trithione treatment in rat submaxillary glands. *J Pharm Pharmacol* 1989; **41**(4): 247-252.

[42] Pedersen-Bjergaard S, Rasmussen KE. Liquid-phase microextraction utilising plant oils as intermediate extraction medium-towards elimination of synthetic organic solvents in sample preparation. *J Sep*

Sci 2004; **27**(17-18): 1511-1516.

[43] Kang P, Kim KY, Lee HS, Min SS, Seol GH. Anti-inflammatory effects of anethole in lipopolysaccharide-induced acute lung injury in mice. *Life Sci* 2013; **93**(24): 955-961.

[44] Ponte EL, Sousa PL, Rocha MV, Soares PM, Coelho-de-Souza AN, Leal-Cardoso JH, et al. Comparative study of the anti-edematogenic effects of anethole and estragole. *Pharmacol Rep* 2012; **64**(4): 984-990.

[45] Conforti F, Tundis R, Marrelli M, Menichini F, Statti GA, De Cindio B, et al. Protective effect of *Pimpinella anisoides* ethanolic extract and its constituents on oxidative damage and its inhibition of nitric oxide in lipopolysaccharide-stimulated RAW 264.7 macrophages. *J Med Food* 2010; **13**(1): 137-141.

[46] Tirapelli CR, de Andrade CR, Cassano AO, De Souza FA, Ambrosio SR, da Costa FB, et al. Antispasmodic and relaxant effects of the hidroalcoholic extract of *Pimpinella anisum* (Apiaceae) on rat anococcygeus smooth muscle. *J Ethnopharmacol* 2007; **110**(1): 23-29.

[47] Albert-Puleo M. Fennel and anise as estrogenic agents. *J Ethnopharmacol* 1980; **2**: 337-344.

[48] Türkyilmaz Z, Karabulut R, Sönmez K, Can Başaklar A. A striking and frequent cause of premature thelarche in children: foeniculum vulgare. *J Pediatr Surg* 2008; **43**(11): 2109-2111.

[49] Tabanca N, Khan SI, Bedir E, Annavarapu S, Willett K, Khan IA, et al. Estrogenic activity of isolated compounds and essential oils of *Pimpinella* species from Turkey, evaluated using a recombinant yeast screen. *Planta Med* 2004; **70**(8): 728-735.

[50] Kreydiyyeh SI, Usta J, Knio K, Markossian S, Dagher S. Aniseed oil increases glucose absorption and reduces urine output in the rat. *Life Sci* 2003; **74**(5): 663-673.

[51] Reddy BS, Rao CV, Rivenson A, Kelloff G. Chemoprevention of colon carcinogenesis by organosulfur compounds. *Cancer Res* 1993; **53**(15): 3493-3498.

[52] Gracza L. [*In vitro* study of the expectorant effect of phenylpropane derivatives of hazelwort. 12. Active substances form *Asarum europaeum*. L]. *Planta Med* 1981; **42**(2): 155-159. German.

[53] Park IK, Choi KS, Kim DH, Choi IH, Kim LS, Bak WC, et al. Fumigant activity of plant essential oils and components from horseradish (*Armoracia rusticana*), anise (*Pimpinella anisum*) and garlic (*Allium sativum*) oils against *Lycoriella ingenua* (Diptera: Sciaridae). *Pest Manag Sci* 2006; **62**(8): 723-728.

[54] Prajapati V, Tripathi AK, Aggarwal KK, Khanuja SPS. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Bioresour Technol* 2005; **96**(16): 1749-1757.

[55] Erler F, Ulug I, Yalcinkaya B. Repellent activity of five essential oils against *Culex pipiens*. *Fitoterapia* 2006; **77**(7-8): 491-494.

[56] Tunç I, Berger BM, Erler F, Dağli F. Ovicidal activity of essential oils from five plants against two stored-product insects. *J Stored Prod Res* 2000; **36**(2): 161-168.

[57] Lee HS. *p*-Anisaldehyde: acaricidal component of *Pimpinella anisum* seed oil against the house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. *Planta Med* 2004; **70**(3): 279-281.

[58] Gilligan NP. The palliation of nausea in hospice and palliative care patients with essential oils of *Pimpinella anisum* (aniseed), *Foeniculum vulgare* var. *dulce* (sweet fennel), *Anthemis nobilis* (Roman chamomile) and *Mentha x piperita* (peppermint). *Int J Aromather* 2005; **15**(4): 163-167.

[59] Picon PD, Picon RV, Costa AF, Sander GB, Amaral KM, Aboy AL, et al. Randomized clinical trial of a phytotherapic compound containing *Pimpinella anisum*, *Foeniculum vulgare*, *Sambucus nigra*, and *Cassia augustifolia* for chronic constipation. *BMC Complement Altern Med* 2010; **10**: 17.

[60] Nahidi F, Taherpoor M, Mojab F, Alavi Majd H. Effect of anise extract on hot flush of menopause. *Pajoohandeh* 2008; **13**(3): 167-173.

[61] Koch C, Reichling J, Kehm R, Sharaf MM, Zentgraf H, Schneele J, et al. Efficacy of anise oil, dwarf-pine oil and chamomile oil against thymidine-kinase-positive and thymidine-kinase-negative herpesviruses. *J Pharm Pharmacol* 2008; **60**(11): 1545-1550.

[62] Tas A. Analgesic effect of *Pimpinella anisum* L. essential oil extract in mice. *Indian Vet J* 2009; **86**(2): 145-147.

[63] Samojlik I, Mijatović V, Petković S, Skrbić B, Božin B. The influence of essential oil of aniseed (*Pimpinella anisum*, L.) on drug effects on the central nervous system. *Fitoterapia* 2012; **83**(8): 1466-1473.

[64] Tas C, Ozkan Y, Okyar A, Savaser A. *In vitro* and *ex vivo* permeation studies of etodolac from hydrophilic gels and effect of terpenes as enhancers. *Drug Deliv* 2007; **14**(7): 453-459.

[65] Schiødt M, Oxholm P, Jacobsen A. Treatment of xerostomia in patients with primary Sjögren's syndrome with sulfarlem. *Scand J Rheumatol Suppl* 1986; **61**: 250-252.

[66] Sangster SA, Caldwell J, Smith RL. Metabolism of anethole. II. Influence of dose size on the route of metabolism of *trans*-anethole in the rat and mouse. *Food Chem Toxicol* 1984; **22**(9): 707-713.

[67] Auerbach SS, Shah RR, Mav D, Smith CS, Walker NJ, Vallant MK, et al. Predicting the hepatocarcinogenic potential of alkenylbenzene flavoring agents using toxicogenomics and machine learning. *Toxicol Appl Pharmacol* 2010; **243**(3): 300-314.

[68] Kwak MK, Huang B, Chang H, Kim JA, Kensler TW. Tissue specific increase of the catalytic subunits of the 26S proteasome by indirect antioxidant dithiolethione in mice: enhanced activity for degradation of abnormal protein. *Life Sci* 2007; **80**(26): 2411-2420.

[69] Coelho-de-Souza AN, Lahlou S, Barreto JE, Yum ME, Oliveira AC, Oliveira HD, et al. Essential oil of *Croton zehntneri* and its major constituent anethole display gastroprotective effect by increasing the surface mucous layer. *Fundam Clin Pharmacol* 2013; **27**(3): 288-298.

[70] Yang JF, Yang CH, Chang HW, Yang CS, Wang SM, Hsieh MC, et al. Chemical composition and antibacterial activities of *Illicium verum* against antibiotic-resistant pathogens. *J Med Food* 2010; **13**(5): 1254-1262.