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REVIEW

Trans-Anethole: A Key Compound in Bogma Raki

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ABSTRACT

Anethole [1-methoxy-4-(1-propenyl)benzene] is a natural flavor commonly used for covering unpleasant odors and in certain food materials and especially in alcoholic drinks. Alcoholic beverages like raki, absinth, mastika include anethole as flavoring agent. Anethole levels are 747-1547 mg/L in nationally certified brands of raki and 441.46-2098.10 mg/L or 0.01-2.6% in bogma raki (illegaly homemade raki) in Turkey. Toxicity and systemic effects of trans-anethole will be discussed in this presentation. Trans-anethole, especially its epoxide metabolite induces the liver cytochrome p450 enzyme. Decreased weight gain, low body weight, anorexia, lethargy, reduction in adiposity, elevated alanine transaminase, aspartate transaminase, gama-glutamyltransferase, and alkaline phosphatase levels, hepatocellular dysmorphology were among effects of trans-anethole observed in animal studies. Inhibition of platelet aggregation as potently as aspirin and antithrombotic activity was revealed in literature. Trans-anethole inhibits pregnancy in a dose-dependent manner because of impaired hormonal balance. Additionally, it was suggested that trans-anethole consumption should be avoided in pregnancy, in breastfeeding mothers or in patients with estrogen-sensitive cancers or endometriosis.
It is suggested that pregnant women, breastfeeding mothers, patients with endometriosis and estrogen-dependent cancers should avoid consumption; furthermore, patients with bleeding disorder, hemophilia, major surgery, and diabetes mellitus should be informed and warned about its toxicity.

Keywords: Anise oil, trans-anethole, alcohol, bogma raki, toxicology

INTRODUCTION

Anethole [1-methoxy-4-(1-propenyl)benzene] is a natural flavor commonly used for covering unpleasant odors and sweetening in baked foods, candies, ice creams, chewing gums and especially in certain alcoholic drinks [1-2]. It exists in essential oils of many plants that grows in the Eastern Mediterranean Region, West Asia, the Middle East, Mexico, Egypt, and Spain such as anise and fennel, members of Apiaceae family, star anise [2-3]. Although it depends on plants' growth area, anethole comprises 76.9–93.7% of essential oil of aniseed [4].
Alcoholic beverages such as raki, absinth, mastika include anethole as flavoring agent [2,5]. Anethole levels range from 747 to 1547 mg/L in nationally certified brands of raki, while it range from 441.46 to 2098.10 mg/L (0.01-2.6%) in bogma raki (illegally homemade raki) in Turkey [6-9]. On the other hand star anise oil which consist of 87% anethole is a popular flavoring agent in confectionary, oral hygiene applications, and particularly medicinal connotations or traditional treatments of diseases [4,10]. Also, the average anethole levels found in nonalcoholic beverages is 42 mg/kg, 53.5 mg/kg in frozen dairy deserts, 531 mg/kg in candies, 495 mg/kg in baked foods, 52.8 mg/kg in gelatins and puddings, and 10 mg/kg in meat and meat products [11].

Chemical Properties and Pharmacokinetics

Physical structure of anethole might be amber liquid or white crystals between 21.3ºC and 234º C. It is highly soluble in ethanol and methanol, slightly soluble in water. Anethole containing liquids turn into a blurry whitish color when it is diluted with water. It is obtained as both cis (also named (Z)) and trans (named (E)) isomers. (E) or trans-anethole is more produced isomer, while (Z) or cis-anethole comprises approximately 0.1-0.7% in plants' essential oils. Although it is accepted as more toxic compared to trans isomer, it's extremely low concentrations in essential oils does not cause any safety concerns [12].

Anethole is absorbed rapidly from gastrointestinal tract after oral administration and undergoes biotransformation in the liver and is removed from body abundantly within 24 hours as 18 different, previously identified, metabolites in urine and biliary excretion [1,12]. Biotransformation of trans-anethole occurs mainly in three pathways: O-demethylation, N-oxidation and epoxidation [1]. In human volunteers carbon labelled trans-anethole was showed to metabolize highly with oxidation and demethylation, while only 3% of given dose was metabolized with epoxidation to anethole 1',2'-epoxide metabolite which is the suspicious agent for hepatotoxicity [13-14].

Systemic Toxicity

a.Gastrointestinal System Toxicity

Trans-anethole, especially epoxide metabolite, induces liver cytochrome p450 enzyme. Increase in activity of CYP1A, CYP2B family members of cytochrome p450 isoenzymes and UDP-glucuronyl transferase, glutathione S-transferase (GST) and DT-diaphorase which play role on phase II reactions was demonstrated following administration of 250 mg/kg trans-anethole [15]. The administration of trans-anethole was showed to cause increase in relative liver weight and expression of cytochrome p450 isoenzymes in a dose dependent manner [16]. Enzyme induction type of trans-anethole resembles to phenobarbital. It induces conjugation and monooxygenase enzymes, and causes proliferation of smooth endoplasmic reticulum, at much higher doses. On the other hand, its prolonged exposure results in increased liver weight [1]. Studies investigating histopathological changes in acute and chronic trans-anethole treated male mice revealed significant hepatocellular hypertrophy, characterized with enlarged heavy livers, due to adaptive physiological alterations secondary to the enzyme-inducing characteristics of trans-anethole [1,17-18]. Sinusoidal dilation, single hepatocellular cell necrosis, infiltration of pigmented macrophages in perivascular region, nodular hyperplasia and hepatocellular carcinoma were also observed at histopathological examination in anethole treated rats and mice [17-18]. Sinusoidal dilation and nodular hyperplasia were showed in trans-anethole treated rats with a concentration higher than 200 mg/kg for 819-847 day, on the other hand hepatocellular carcinoma occurred after administration of 550 mg/kg trans-anethole for 819-847 day, in 6 out of 52 female rats [18]. Alanine transaminase, aspartate transaminase, gama-glutamyltransferase and alkaline phosphatase values were elevated following administration of 120 mg/kg or more [17]. Controversially Newbern et al. stated that the frequency of histopathological findings including hepatocellular carcinoma and biochemical alterations was not significantly different than control group and might be incidental findings and alterations, except hepatocellular hypertrophy occurred seconder to enzyme induction [1].

A concentration and exposure time dependent cell death was shown in rat hepatocytes directly exposed to trans-anethole (0.25–2.0 mM), which was accompanied by depleted intracellular ATP and total adenine nucleotides. These exposure consequently causes surface blebs formation and morphological damage of cell [19].

Decreased body weight gain, low body weight, anorexia, lethargy, reduction in adiposity that observed in animals administered 1% trans-anethole were attributed to the decrease in food intake [18].

b.Cardiovascular System Toxicity

Anethole affected voltage dependent calcium channel and cause opening directly or indirectly at micromolar concentrations that consequently results in vascular smooth muscle contraction in rats aorta [20]. On the other hand, vasorelaxant properties was demonstrated by Tognolini at al. in thoracic aorta of rats [21].

Foeniculum vulgare which contains 75.8% trans-anethole causes arachidonic acid-induced platelet aggregation in the study by Tognolini et al. [22]. Besides inhibition of platelet aggregation as potently as aspirin, antithrombotic activity was shown in mice treated with oral doses of 30 mg/kg/day for five days [21].

Anethole 1',2'-epoxide, a minor reactive metabolite of trans-anethole, depletes glutathione and causes cytotoxicity due to increased reactive oxygen species [14]. The toxicity of trans-anethole related to anethole 1',2'-epoxide is accepted safe because of

low amount of production after biotransformation [1,11].

Anise oil contained 76.7 ± 12.79% of trans-anethole provokes the glucose absorption accompanied by elevated Na/K ATPase activity [23]. Nevertheless, in 72.6% trans-anethole containing fennel oil treated rats decreased glucose levels were observed [24]. Trans-anethole causes decrease in plasma glucose levels and glycosylated haemoglobin (HbA1c), while it increases insulin and haemoglobin levels. A study dealing with effects of trans-anethole -at doses of 20-40-80 mg/kg/day for 45 days- in streptozotocin induced diabetic rats normalization of glucose metabolism enzyme levels in liver and kidney, improved hepatic and muscle glycogen content, amelioration of pancreas B-cells were demonstrated [25]. Despite controversial results, it is recommended that patients on antidiabetic medications must be careful in respect of consumption of trans-anethole containing food and beverages [11].

c.Reproductive Toxicity

Fennel, anise oil and their main component trans-anethole were studied in terms of estrogenic activity, which revealed slight positive responses [26]. This weak estrogenic activity was linked to a polymer or a metabolite rather than the compound itself [27]. Nakagawa et al. examined estrogenic activity of anethole in MCF-7 human breast cancer cell line which express estrogen receptors and sensitive to estrogenic activity for proliferation. Trans-anethole and 4MCA (4-methoxycinnamic acid), an oxidation metabolite of trans-anethole, showed weak or negligible estrogenic activity, while 4OHBP (4-hydroxy-propenylbenzene), an O-demethylation metabolite, has competitive agonism to estrogen receptors resembling 17b-estradiol [19]. The activity potency of studied molecules was ordered as diethylstilbestrol > 4OHPB > 4MCA > anethole. 4OHPB loses its affinity to estrogenic receptors when derived with sulphate and glucuronide [19]. Although estrogenic activity of trans-anethole was indicated extremely low or negligible by Nagakawa et al., Tabanca et al. showed that estrogenic activity of trans-anethole was 54.4% of 17b-estradiol in Saccharomyces cerevisiae culture that expresses the human estrogen receptor alpha [28]. The results of the study by Tabanca et al. were compatible with the study by Howes et al. using similar method [29]. Dhar et al. studied the effects of trans-anethole on fertilization in rats treated with 50-70-80 mg/kg/day for 1-10 days. They observed dose dependent inhibition of pregnancy because of hampered implantation based on impaired hormonal balance via estrogenic activity of trans-anethole. Although no gross malformation was observed in pups, abortifacient activity was seen in the early period of pregnancy [30]. Otherwise in trans-anethole treated male and females mated pairs to produce second, third and fourth generation, pups had low birth weight and difficulty in weight gain at doses of 700 mg/kg/day [1,31]. However, similar findings were shown at 175 and 350 mg/kg dose in rats. On the other hand, rats treated with 350 mg/kg showed mild increase in gestation time, pup mortality, and stillbirths [1,32]. Ostad et al. investigated the effects of fennel oil containing 72% trans-anethole for teratogenicity and developmental differentiations at limb buds. Though there was no evidence of teratogenicity, reduction in differentiation of limb buds was related to cytotoxic effects rather than inhibition of differentiation, in rat embryos [33].

Trans-anethole administration induces significant increase of the uterine weight [30]. Furthermore fennel oil containing 72% of trans-anethole causes inhibition of uterine contraction [34]. Also trans-anethole reaches detectable levels in human breast milk at 2nd hour of oral administration until 8th hour of post-ingestion [35].

It was suggested that trans-anethole consumption should be avoided in pregnancy, in breastfeeding mothers or in patients with estrogen-sensitive cancers or endometriosis [11].

d.Central Nervous System

Fennel oil use related generalized tonic-clonic epileptic seizure was described in a case report in which the patient's epilepsy treated with lamotrigine. Patient suffered from unconsciousness for 45 minutes after five-six, homemade, unknown amount of fennel oil containing cake consumption [36]. Although cause of seizure was attributed to primary neurotoxicity of fennel oil, another possible explanation might be trans-anethole's enzyme induction activity accelerating the metabolism of lamotrigine [15]. On the other hand, anise oil containing 89.1% of trans-anethole was shown to prolong seizure latency and discharge, while it reduces the amplitude and duration of epileptiform burst discharges in epileptic seizures induced by pentylenetetrazol [37]. Studies by Abdul-ghani et al. and Pourgholami et al. showed that trans-anethole containing plants had anti-convulsant effect, and protective effects for hypoxic conditions due to anti-excitotoxicity activity of trans-anethole [38-40].

Trans-anethole inhibits acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes and IC50 values for AChE and BChE are 39.89±0.32 µg/mL and 75.35±1.47 µg/mL, respectively [41].

Genotoxic and Carcinogenic Effects

Mutagenic effect of trans-anethole is well documented. Positive mutagenic results were obtained from Ames test in Salmonella Typhimurium TA98, TA100, TA1535, TA1537, TA1538 strains with S9, S13 activation and cofactor PAPS addition, mouse lymphoma assay in mouse lymphoma L5178 cell line with S13 activation, chromosomal aberration test in Chinese hamster ovary cell line with S13 activation [42-47]. However, in some other strains negative results were also observed via Ames test, chromosomal aberration test, and mouse lymphoma assay without any activation [42-47]. Muller et al. indicated slight increase in UDS in rat hepatocyte cell culture via DNA repair test [48]. Additionally negative results were demonstrated via DNA post-labelling assay, micronucleus assay, DNA repair test (UDS, rec assay), uvrA reversion test in *Salmonella typhimurium*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Escherichia coli* strains, rat hepatocyte cultures, rats, and mice [14,46,49-55]. Trans-anethole epoxide had no genotoxic effects in rat hepatocyte culture via DNA repair test (UDS) in the study by Marshall and Caldwell. Kim et al. found out positive Ames test with point and frameshift mutations in *Salmonella typhimurium* strains [52,56]. Therefore, authors emphasized that trans-anethole is not genotoxic, without regard to mutagenic activity of epoxide metabolite due to low amounts of production [1,11].

After administration of trans-anethole at different doses (148-370-740 mg/kg, twice weekly, for 10-12 week, 703-1390 µg/kg, ip, on 1, 8, 15 and 22 after birth, 690 mg/kg/day for 12 months) no increase was observed the incidence of hepatic tumors in mice [1,57]. However, this finding was considered as inadequate due to failure of completion of standard 2-year bioassay [1]. On the other hand, Truhaut et al. indicated that slight but statistically significant increases of hepatocellular adenoma and carcinoma incidences were observed at their study lasting for 117-121 weeks on 550 mg/kg/day of trans-anethole administered rats [18]. This study was reassessed by Newbern et al. and results were confirmed [58]. A study by Auerbach et al. about hepatocarcinogenic gene expression of rats exposed to trans-anethole at 0,2 or 2 mmol/kg/day for 2, 14 or 90 days showed that trans-anethole can be classified among non-hepatocarcinogens [59]. Trans-anethole epoxide has remained at suspicious side since it might cause hepatoma and skin papilloma. This data suggests that in vivo epoxidation of trans-anethole could be responsible for its toxicity and carcinogenicity [56].

Low doses of trans-anethole have negligible risk for carcinogenicity, in human. However, current studies indicated that trans-anethole has anticarcinogenic and genoprotective effects [11].

Acute Effects

Oral and intraperitoneal LD50 doses of trans-anethole are between 1820 mg/kg and 5000 mg/kg [1]. Administration of 695 mg/kg dose for 4 days, acute liver injuries such as discoloration, mottling and blunting of lobe edges were observed [1,60]. Otherwise administration of 60-120 mg/kg/day for 90 days caused to inanition syndrome due to decreased food and water consumption starting on 3rd or 4th week of treatment. Decreased liver glycogen content and depletion of total organ weight correlated with decreased cellularity observed at 30-60 mg/kg/day for 90 days [1,61]. In mice studies, deaths were observed at concentrations in the diet 120, 240, 360, and 500 mg/kg/day, as a result of starvation and dehydration. On the contrary, in rat studies, no deaths occurred with administration of 1200 mg/kg/day [62]. Based on necrosis findings and gama-glutamyltransferase alterations, NOAEL (No Observed Adverse Effect Level) were determined at 300 mg/kg/day [61]. On the other hand NOAEL obtained from the studies lasting more than 2 years was concluded to be 120 mg/kg/day [1,18,58].

The level of LD50 was 1326 mg/kg for fennel essential oil. The literature reveals that sedation, respiratory distress, movement disorder, and unresponsiveness to external stimulus, limb weakness, tremor and muscles' fasciculation were observed within 24 hours after administration, while no significant tissue damage was detectable histopathologically [34].

Adverse skin reactions such as contact dermatitis, dermal papilloma, skin sensitization, cheilitis were reported in certain studies [56,63-65].

Trans-anethole toxicity is highly related to trans-anethole epoxide in a dose-dependent manner. Epoxidation rate differs from species to species, and it is higher in rats than mice, while it is least in human. The NOAEL of 120 mg trans-anethole/kg/day corresponds to production of approximately 22 mg AE/kg/day in rats, which is more than 10 000 times the level of 0.002 mg AE/kg/day produced by human. This is considered as explanation for lesser hepatotoxic effect in human compared to animal models [1]. Acceptable Daily Intake (ADI) level was assigned to be 2 mg/kg by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1998 [66].

CONCLUSION

Trans-anethole is a common flavoring agent in alcoholic, non-alcoholic beverages and foods. It is suggested that pregnant women, breastfeeding mothers, patients with endometriosis and estrogen-dependent cancers should avoid consumption, while patients with bleeding disorder, hemophilia, major surgery, childbirth, controlled diabetes mellitus should be informed and warned about its toxicity [11].

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