Anti-inflammatory and Hypoglycemic Activities of Alpha-pinene

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ABSTRACT

The aim of this study is to investigate the anti-inflammatory and hypoglycemic activities of alpha-pinene, and to find the median lethal dose (LD_{50}) level in mice.

Lethal dose levels of alpha-pinene were investigated using a probit analysis method. For the anti-inflammatory activity measurement seven different groups were established and alpha-pinene was administered in four different doses: 0.05, 0.10, 0.25 and 0.50 mL/kg. For the evaluation of hypoglycemic activity six different groups, consisting of diabetic and healthy mice, were established.

The strongest anti-inflammatory activity of alpha-pinene was observed with a 0.50 mL/kg dosage. The median effective dose (ED_{50}) value of alpha-pinene was found to be 0.039 mL/kg. In diabetic mice α-pinene showed significant levels of hypoglycemic activity at the 2nd and 24th hours. LD_{50} level of α-pinene was determined to be 2.076 mL/kg.

As a result, we conclude that alpha-pinene is a molecule that displays hypoglycemic and anti-inflammatory activities.

Keywords: Alpha-pinene, anti-inflammatory activity, hypoglycemic activity, mice, rats.

INTRODUCTION

Foeniculum vulgare Miller, (family Umbelliferae) is an annual, biennial or perennial aromatic herb, depending on the variety, which has been known since antiquity in Europe and Asia Minor. The leaves, stalks and seeds (fruits) of the plant are edible[1]. Extracts of *Foeniculum vulgare* Miller (fennel) seeds are used as an anti-inflammatory agent in Turkish traditional medicine[2]. The anti-inflammatory, hypoglycemic and hepatoprotective effects of fennel were dem-

onstrated in previous studies [3-7]. The volatile components of fennel seed extracts are *trans*-anethole, fenchone, methylchavicol, limonene, alpha-pinene, camphene, β-pinene, β-myrcene, α-phellandrene, 3-carene, camphor, and cisanethole [8]. The major components of the fennel seed essential oil are alpha-pinene, limonene, fenchone, methychavicol and *trans*-anethole[6].

In our previous work, we demonstrated the anti-inflammatory and hypoglycemic activities of volatile oil extract of fennel[3-7]. In order to determine the fennel component(s) responsible for these activities and to determine its lethal dose levels, this study investigates alpha-pinene, a major component of volatile oil extract of fennel.

METHODOLOGY

Animals

Sprague-Dawley rats and Mus musculus Swiss albino mice were maintained in the animal house. The animals were housed in standard cages with pelleted food and water *ad libitum*, at room temperature (22±2 °C) with a 12h light-dark cycle. Ethical approval was obtained from the Animal Ethics Committee.

Chemicals

(1R)-(+)-α-pinene ($C_{10}H_{16}$), lambda-carrageenan Type IV, indomethacin and alloxan were obtained from Sigma (Steinheim, Germany), and glibenclamide was obtained from Nobel (Istanbul, Turkey).

Acute toxicity

Swiss albino mice were randomly assigned to nine groups with six animals in each group. The control group was treated with isotonic saline solution (ISS) (0.9% NaCl), and the other eight groups were treated with alpha-pinene given intraperitoneally (ip) by Hamilton and insulin injectors in increasing dosages of 0.05, 0.10, 0.20, 0.40, 0.80, 1.60, 2.40 and 3.20 mL/kg body weight. The mortality in each cage was assessed 72 h after administration of alpha-pinene. The percentage mortalities were converted to probits. Regression lines were fitted by the method of least squares and confidence limits for the LD_1, LD_{10}, LD_{50}, LD_{90} and LD_{99} values were calculated by the method of Litchfield & Wilcoxon[9] and Kouadio et al[10].

Anti-inflammatory activity

The method of Winter et al was used with slight modification[11]. Forty-two rats were divided into seven groups of six animals each. The rats were starved for 12 h and deprived of water only during the experiment. Water deprivation was em-

ployed to ensure uniform hydration and to minimize variability in edematous response. Inflammation of the hind paw was induced by injecting 0.05 mL of fresh lambda carrageenan (phlogistic agent) into the subplantar surface of the right hind paw. The control group-I was given ISS (0.1 mL) and the control group-II was given ethyl alcohol (0.1 mL). The third group (reference group) received the anti-inflammatory agent indomethacin (3 mg/kg, ip), while the remaining four groups received alpha-pinene at doses of 0.05, 0.10, 0.25 and 0.50 mL/kg, i.p by Hamilton injector[12]. The doses utilized in the current study were chosen according to LD_1 value (LD_1 = 0.744 mL/kg).

Foot volume was measured by a displacement technique using a plethysmometer (Ugo Basile 7140 plethysmometer, Italy), immediately before and three hours after the 0.05 mL of fresh lambda carrageenan injection. The percentage inhibition of the inflammatory reaction was determined for each animal by comparison with controls and calculated by the formula[10]:

I % = [(1-(dt/dc)] x 100

where *dt* is the difference in paw volume in the drug-treated group and *dc* the difference in paw volume in the control group.

Preparation of alloxan diabetic mice

Diabetes was induced by i.p. injections of 150 mg/kg alloxan monohydrate prepared in ISS three times with 48 h intervals. Before injections were given mice were starved for 18 h[13]. Seven days after the last injection, fasting blood glucose levels were measured and mice with fasting blood glucose levels of 200 mg/dL and over were taken into the study[14].

Hypoglycemic activity in normal and diabetic mice

Diabetic animals were randomly divided into three groups of six animals each. Group I mice received 0.1 mL ISS i.p. The animals in group II were treated orally with 3.0 mg/kg glibenclamide, a hypoglycemic agent, used as reference. Group III received i.p. injection of 0.25 mL/kg alpha-pinene by Hamilton injector. The same protocol described above was applied in three groups of normal mice. Fasting blood glucose levels were measured after 18 h of fasting just before the treatment and 1, 2, 4 and 24 h after the treatment using the glucose oxidase peroxidase method (Abbott, United Kingdom).

Statistical analysis

Results were reported as mean ± standard error of mean (SEM). The total variation was analyzed by performing a one-way analysis of variance (ANOVA). Least Significant Difference (LSD) test, Dunnet test and Tukey's HSD (Honestly

Significant Difference) test were used for determining significance. Probability levels of less than 0.05 were considered significant. The medium effective dose (ED_{50}) value was calculated by non-linear regression analysis (SigmaPlot 2004 for Windows Version 9.01).

RESULTS AND DISCUSSION

Acute toxicity

The lethal doses of alpha-pinene are presented in Table 1. The intraperitoneal medium lethal dose (LD_{50}) value for the total number of animals was found to be 2.076 mL/kg.

Table 1. Lethal doses of (1R)-(+)-α-pinene ($C_{10}H_{16}$).

	Dose (mL/kg)	95% confidence limits	
Lethal doses		Lower (mL/kg)	Upper (mL/kg)
LD_1	0.744	0.012	1.242
LD_{10}	1.179	0.111	1.663
LD_{50}	2.076	1.246	3.180
LD_{90}	3.655	2.630	32.295
LD_{99}	4.497	3.023	86.301

Anti-inflammatory activity

Table 2 shows the anti-inflammatory effects of intraperitoneally administered alpha-pinene on carrageenan induced paw edema in rats. Alpha-pinene showed significant anti-inflammatory effect in two doses studied (0.025 mL/kg and 0.50 mL/kg); peak response was obtained with 0.50 mL/kg alpha-pinene (60.33% decrease in inflammation) and 0.05 mL/kg alpha-pinene caused a lesser degree of inhibition of the inflammation (18.97%). Compared to the controls, the greatest anti-inflammatory activity was observed in the indomethacin group, with a 87.44% regression of the inflammation. Compared to indomethacin group, alpha-pinene group had significantly lower anti-inflammatory effects at all doses. At 0.05 mL/kg dose the alpha-pinene group showed significantly lower anti-inflammatory activity compared to the 0.50 mL/kg dose. The medium effective dose (ED_{50}) value of alpha-pinene was found to be 0.039 mL/kg.

Table 2. Effects of alpha-pinene on rat paw edema.

Groups	Dose	Paw edema (mL %)	Inhibition (%)
Control-I (ISS)	0.1 mL	1.043 ± 0.127	-
Control-II (ethyl alcohol)	0.1 mL	0.988 ± 0.112	-
Indomethacin	3 mg/kg	[ab] 0.024 ± 0.061	87.44
Alpha-pinene	0.05 mL/kg	[c] 0.845 ± 0.109	18.97
Alpha-pinene	0.10 mL/kg	[c] 0.672 ± 0.051	35.57
Alpha-pinene	0.25 mL/kg	[abc] 0.617 ± 0.073	40.83
Alpha-pinene	0.50 mL/kg	[abcd] 0.413 ± 0.069	60.34
F value		*17.750*	
p value		*0.000*	

Data is presented as mean ± standard error of the mean (*n*=6).

ED_{50}: 0.039 mL/kg.

Post-hoc Tukey's HSD and Dunnet tests:

a : *p<0.05* compared to control-I (ISS) group,

b : *p<0.05* compared to control-II (ethyl alcohol) group,

c : *p<0.05* compared to indomethacin group,

d : *p<0.05* compared to alpha-pinene 0.05 mL/kg.

Hypoglycemic activity

The fasting blood glucose levels of the alloxan diabetic mice are presented in Table 3. Table 4 demonstrate the levels of fasting blood glucose in normal mice. It was determined that alpha-pinene significantly decreased fasting blood glucose levels at the 2nd and 24th hours. It was observed that alpha-pinene significantly increased fasting blood glucose levels in healthy mice at the 1st and 2nd hours.

Table 3. Effects of alpha-pinene on fasting blood glucose levels in diabetic mice.

Groups	Fasting blood glucose (mg/dL)				
	Before treatment	**1st hour**	**2nd hour**	**4th hour**	**24th hour**
Control (ISS)	337.2±23.4	318.4±25.3	308.0±34.2	225.0±34.4	205.4±19.3
Glibenclamide	267.3±37.7	197.8±47.3	[a] 150.5±39.7	[a] 101.8±10.6	[a] 90.1±15.4
Alpha-pinene	290.4±17.4	306.1±37.0	[a] 196.1±26.3	[b] 170.7±25.5	[a] 137.6±31.4
F values	*1.539*	*2.827*	*5.291*	*5.786*	*6.865*
p values	*0.247*	*0.091*	*0.018*	*0.014*	*0.009*

Data is represented as mean ± standart error of the mean.

Post-hoc LSD test:

a: p<0.05 compared to ISS group.

b: p<0.05 compared to glibenclamide group.

Table 4. Effects of alpha-pinene on fasting blood glucose levels in healthy mice.

Groups	Fasting blood glucose (mg/dL)				
	Before treatment	1st hour	2nd hour	4th hour	24th hour
Control (ISS)	91.50±12.8	72.75±7.2	60.50±4.1	61.25±4.1	54.50±3.0
Glibenclamide	a 68.75±01.3	59.25±4.8	59.00±3.6	53.25±2.9	49.75±2.0
Alpha-pinene	b 96.0±03.8	ab 102.3±10.8	ab 79.6±4.2	b 76.6±7.3	73.5±11.7
F values	*4.274*	*6.019*	*8.155*	*4.059*	*2.060*
P values	*0.042*	*0.017*	*0.007*	*0.048*	*0.174*

Data is represented as mean ± standart error of the mean.

Post-hoc LSD test:

a: p<0.05 compared to ISS group.

b: p<0.05 compared to glibenclamide group.

In this work, the LD_{50} dose of alpha-pinene, a major component of the essential oil of *Foeniculum vulgare* Mill. was determined to be 2.076 mL/kg.

The current study clearly demonstrated the in vivo anti-inflammatory effect of alpha-pinene at doses of 0.25 mL/kg and 0.50 mL/kg. The ED_{50} dose of alpha-pinene related to its anti-inflammatory activity was found to be 0.039 mL/kg for its.

It has been reported that *Bupleurum fruticescens*, Salvia species and Helichyrisum species essential oils had anti-inflammtory effects. The anti-inflammatory activity shown by the essential oil can be attributed to the two major components-alpha-pinene and beta-caryophyllene[15-17]. The results of these studies are consistent with those of the current study. Zhou et al. reported that alpha-pinene inhibits the nuclear translocation of NF-kappa B induced by lipopolysaccharide (LPS) in THP-1 cells[18]. The transcription factor NF-kappa B plays a pivotal role in the activation of multiple inflammatory molecules[19]. Kim et al claimed that alpha-pinene exhibits anti-inflammatory activity through the suppression of mitogen-activated protein kinases (MAPKs) and the nuclear factor-kappa B (NF-κB) pathway in mouse peritoneal macrophages[20]. It can be suggested that alpha-pinene shows its anti-inflammatory effect through the NF-kappa B. Rufino et al. reported that at noncytotoxic concentrations, (+)-α-pinene elicited the most potent inhibition of the IL-1β-induced inflammatory and catabolic pathways, namely, NF-κB and JNK activation and the expression of the inflammatory (iNOS) and catabolic (MMP-1 and -13) genes in human chondrocytes[21].

It is found that alpha-pinene increased fasting blood glucose levels in healthy mice significantly at the 1st and 2nd hours. However, since these values are within normal limits the increments in fasting blood glucose levels induced by alpha-pinene, these observed values are considered to have no clinical significance. In addition, the results show that alpha-pinene caused mild hypoglycemic activity in the diabetic mice at the 2nd and 24th hours investigating. There is no study of the hypoglycemic activity of alpha-pinene in the literature. For this reason, it is not possible to comment about the mechanism of its the hypoglycemic activity. Further investigations must be conducted to reveal the mechanisms of the anti-inflammatory and hypoglycemic activities of alpha-pinene.

In conclusion, the current study shows that alpha-pinene had anti-inflammatory and hypoglycemic activities in vivo.

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