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Effect of an aqueous distillate of *Origanum onites* L. on isolated rat fundus, duodenum and ileum: Evidence for the role of oxygenated monoterpenes

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The aqueous distillate of *Origanum onites* L. (Labiatae) was reported to have various ethnomedical uses including effects on the gastrointestinal tract. The effects of three different doses (0.1, 0.2 and 0.4 mL) of the aqueous distillate of *Origanum onites* on isolated rat fundus, duodenum and ileum against acetylcholine induced contractions were investigated in this study. The aqueous distillate inhibited contractions in a dose dependent manner. Inhibitions were lowest on fundus. Carvacrol, being the major compound of the test substance, did not inhibit acetylcholine induced contractions of the isolated rat fundus, indicating the presence of other active principles including menthane diols. As being one of the first pharmacological studies on aqueous distillates, a possible pharmacological activity of *cis-p*-menth-4-ene-1,2-diol and 3,7-dimethyl-1-octen-3,7-diol is proposed in this study.

1. Introduction

Origanum onites (Labiatae) is a plant traditionally used for various purposes. More than one *Origanum* species is used ethnomedically (Baytop 1984) and the ethnomedical use of *Origanum* species dates back to antique days of Hippocrates (Aydin et al. 1993a; Skrubis 1979; Demirhan 1974; Holtam and Hylton 1979) and mediavel ages (Lev 2002) as an antidote against insect and animal bites and poisons (Aydin et al. 1993a; Skrubis 1979; Demirhan 1974), infections (Baytop 1984; Lev 2002), pain (Aydin et al. 1993a; Demirhan 1974; Lev 2002; Bonet et al. 1992), cough (Demirhan 1974; Boukef et al. 1982), disorders of urinary and genital organs (Demirhan 1974; de Laszlo and Henshaw 1954; Dragendorff 1898; Tabata et al. 1994; Jochle 1974), diabetes (Demirhan 1974) hepatitis (Tabata et al. 1994), epilepsia (Tabata et al. 1994), healing wounds and against tumors (Hartwell 1982; Rios et al. 1987), rheumatism (Aydin et al. 1993a) and gastrointestinal disturbances (Aydin et al. 1993a; Lev 2002; Tabata et al. 1994). Recently *O. onites* gained commercial importance and the chemical composition of the essential oils of commercially exported species were studied (Baser et al. 1993). In addition to volatile oils (Skoula et al. 1999), the presence of other compounds including glycosidically bound volatile oils (Stahl-Biskup et al. 1993), lipids (Azcan et al. 2000), diterpenes (Passannanti et al. 1984) triterpenes (Hegnauer 1966; Hegnauer 1989; Piozzi et al. 1986) and flavonoids are shown in Origanum onites (Thomas-Barberan et al. 1988). Although ethnomedically used for many centuries its chemical composition and pharmacological properties began to be investigated recently (Aydin et al. 1996a).

An aqueous distillate only contains a limited number of oxygenated volatile molecules (Table 1) (Aydin et al. 1996a) whereas an aqueous extract may contain flavonoids, saponins triterpenes and tannins (Desmarchelier et al. 2001). The aqueous distillate of *O. onites* (ADO), traditionally obtained using a simple household apparatus in a single step (Aydin et al. 1993a) is nontoxic (Aydin et al. 1996a). Among the initial pharmacological results were lack of blood pressure lowering effects, no toxicity in acute and chronic toxicity models, hepatoprotective action against carbontetrachloride induced toxicity (Aydin et al. 1993b). Its sedative activity and augmentation of barbiturate sleeping time indicated the central nervous system as an important target (Aydin et al. 1993b, 1996a, 1996b). Although the essential oil of the plant showed analgesic activity, ADO was devoid of this effect (Aydin et al. 1996b).

Taking into consideration the traditional use and ethnopharmacological data, an investigation of ADO on gastrointestinal smooth muscles was performed in this study.

2. Investigations, results and discussion

The aqueous distillate of *Origanum onites* L. (ADO) was tested on isolated rat fundus, duodenum and ileum at three different doses (0.1, 0.2 and 0.4 mL) of the test substance. Acetylcholine induced contractions were observed to be inhibited in a non-competitive manner on fundus (Fig. 1), duodenum (Fig. 2) and ileum (Fig. 3). Inhibitory effect was low on fundus. The % response against 10^{-5} M acetylcholine in the presence of 0.4 mL ADO was 42.67 ± 6.42, 23.47 ± 3.83 and 15.76 ± 8.94 on fundus, duodenum and fundus, respectively (Figs. 1–4). The inhibitions were also

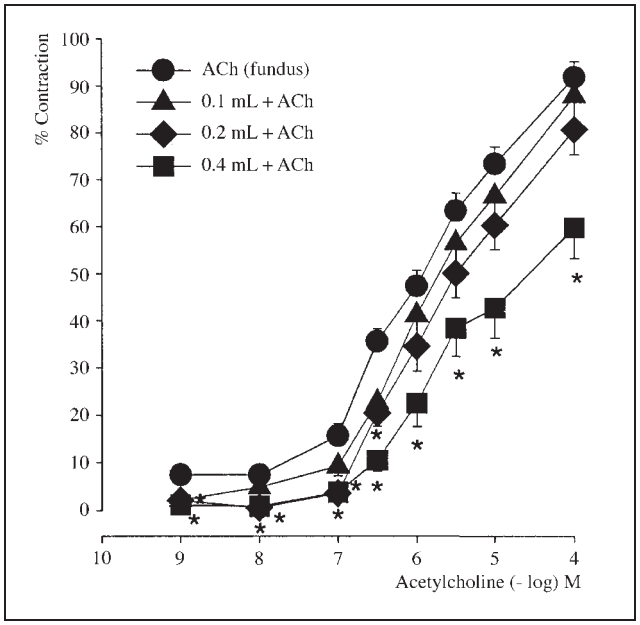


Fig. 1: Effect of three different doses of aqueous distillate of *Origanum onites* on isolated rat fundus. ACh = acetylcholine. Values are expressed as mean ± s.e.mean (*) $p < 0.05$. (n = 10)

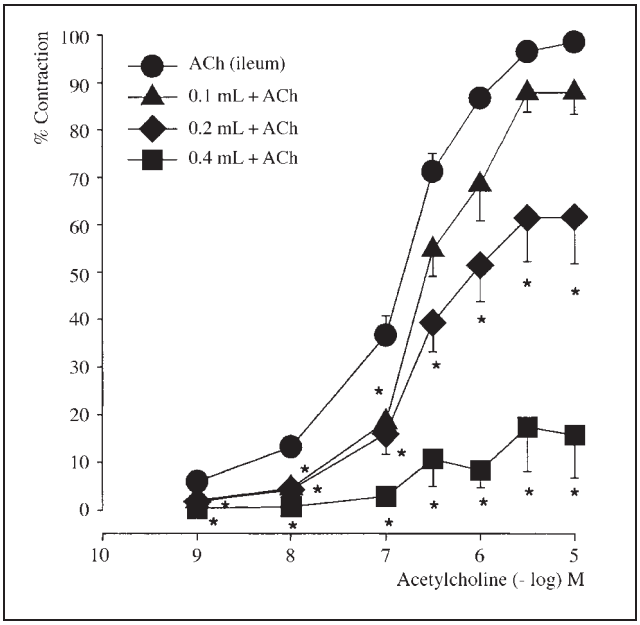


Fig. 3: Effect of three different doses of aqueous distillate of *Origanum onites* on isolated rat ileum. ACh = acetylcholine. Values are expressed as mean ± s.e.mean (*) $p < 0.05$. (n = 10)

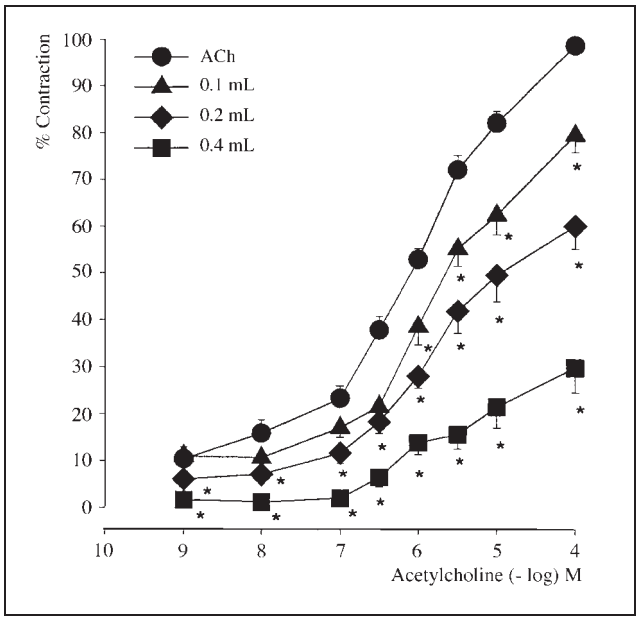


Fig. 2: Effect of three different doses of aqueous distillate of *Origanum onites* on isolated rat duodenum. ACh=acetylcholine. Values are expressed as mean ± s.e.mean (*) $p < 0.05$. (n = 10)

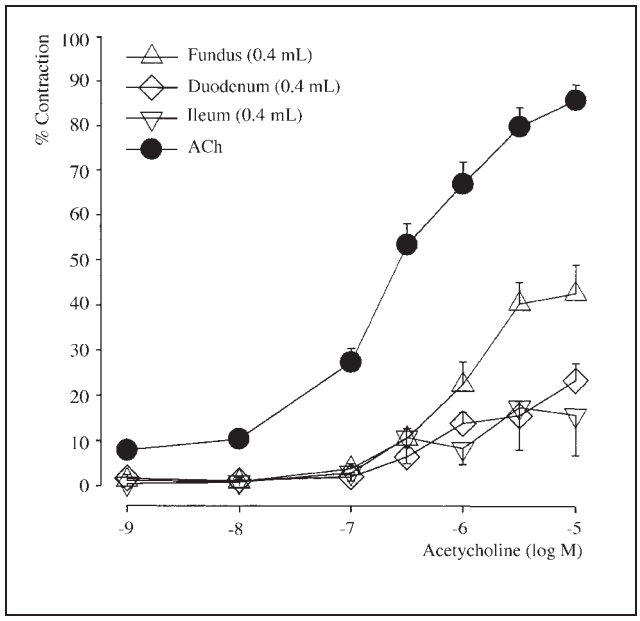


Fig. 4: Comparison of the effects of highest dose of aqueous distillate of *Origanum onites* (10^{-4} mL) on isolated rat fundus, and ileum. ACh = acetylcholine. Values are expressed as mean ± s.e.mean (n = 10). Multiple comparison for these groups were not performed therefore differences are not shown by (*)

observed to increase towards distal of the gastrointestinal system. Inhibition of KCl induced contractions of isolated rat ileum by ADO was previously reported (Aydin et al. 1996a) but to the best of our knowledge, inhibition of acetylcholine induced contractions on ileum, duodenum and fundus was not reported to date. Moreover tissue specific differences were noticed on the inhibitions, being fundus the least inhibited but ileum was the most sensitive organ to the effect of ADO (Fig. 4).

Since carvacrol was the major compound, it was hypothetized to be the active ingredient. Considerable low amounts of other compounds in ADO were also another factor for this hypothesis (Table) (Aydin et al. 1996a). In order to test this hypothesis, carvacrol was tested at three doses (10^{-6}, 10^{-5} and 10^{-4} M) on the isolated rat fundus. Surprisingly no significant inhibition was observed with pure carvacrol. Concentration of carvacrol in the aqueous distillate was calculated not to exceed 10^{-5} M in ADO, but the lack of significant activity of pure carvacrol (even at the dose of 10^{-4} M) showed that carvacrol itself was not enough to exert an activity on the isolated rat fundus (Fig. 1). Apparently our hypothesis regarding the effect of carvacrol on acetylcholine induced contractions of the isolated rat fundus failed.

This experimental data led us to think on other mono- and dioxygenated compounds that are previously shown to be present in the ADO (Aydin et al. 1996a). The only explanation for this experimental discrepancy is an activity of

Table 1: Composition of the aqueous distillate of *Origanum onites* L. (ADO) (Aydin et al. 1996a)

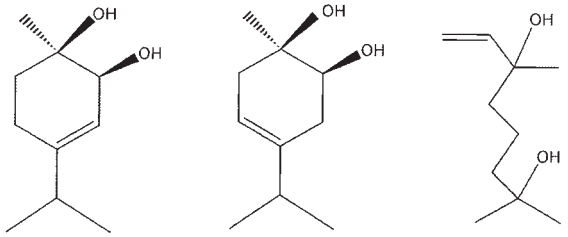
Compound	SPME (*)	Chloroform fraction	Hexane fraction
Linalool	5.55	0.55	3.35
Terpinen-4-ol	1.45	0.26	1.83
α-Terpineol	0.59	0.90	4.65
Borneol	1.61	0.48	2.66
3,7-Dimethyl-1-octen-3,7-diol	0.15	21.03	0.34
cis-p-Menthan-1,8-diol	–	6.22	0.01
3,7-Dimethyl-1,7-octadien-3,6-diol	0.10	1.61	–
cis-p-Menth-4-ene-1,2-diol	8.71	15.59	1.15
cis-*p*-Menth-3-ene-1,2-diol	0.78	1.57	–
Thymol	9.56	1.47	6.10
Carvacrol	63.76	26.24	73.01

(*) SPME = Solid-phase micro extraction

newly defined dioxygenated compounds and some of the known oxygenated monoterpenes present in ADO.

The importance of the role of the hydroxyl group for the activity is shown for carvacrol (van den Broucke and Lemli 1980; Ultee et al. 2002) and for other compounds (Aydin et al. 2003). Although carvacrol was shown to possess various activities on bacteria (Lambert et al. 2001), fungi (Manohar et al. 2001), mosquitos (Traboulsi et al. 2002), tumors (Zeytinoglu et al. 1998) and on the central nervous system (Aydin et al. 1996a, Vanderwolf et al. 2002), we suggest that other oxygenated compounds of ADO are responsible for the observed effects.

Dioxygenated compounds of the test substance, like *cis-p*-menth-3-ene-1,2-diol (**1**), cis-*p*-menth-4-ene-1,2-diol (**2**) and 3,7-dimethyl-1-octen-3,7-diol (**3**) do not appear in essential oils. To the best of our knowledge, these diols have not been subject to any pharmacological studies.



Although one more phytochemical study was reported mean while (Bogdag et al. 2000), these compounds deserve much more attention and must be taken into consideration for further chemical and pharmacological investigations. To the best of our knowledge, our investigation is the first to emphesize the putative pharmacological activity of *p*-menth-diols found in aqueous distillates but not in essential oils. Based on the present results, aqueous distillates of other plant species remain to be investigated as possible new sources of pharmacologically active compounds.

3. Experimental

3.1. Animals test materials and chemicals

Adult albino Wistar rats (185–260 g) of either sex were used in this study. They were housed in well ventilated rooms with a room temperature of 18–25 °C. All animals were fed with standard diet (Esyem A.S., Eskisehir) and water *ad libitum*. The test material was obtained from *Origanum onites* L. of West Anatolian origin and the aqueous distillate was obtained by hydrodistillation, which accumulated under the essential oil (Aydin et al. 1996a). The test material was kept in dark glassware in room temperature in order to protect it from light, as advised by the villagers (Aydin et al. 1993a). Acetylcholine chloride, carvacrol (Sigma, St.Louis, MO) and dimethyl sulfoxide (DMSO) were obtained commercially (Merck, Schuchardt, Germany).

3.2. Isolated organ bath experiments

Rats were killed by stunning and decapitation. The organs (fundus, duodenum and ileum) were then excised from each animal and kept in Krebs' solution with the following composition (in mM): NaCl, 118,4; KCl, 4,7; $CaCl_2 \cdot 2\,H_2O$, 1.9; $NaHCO_3$, 25.0; $MgSO_4 \cdot 7\,H_2O$, 1.2; KH_2PO_4, 1.2 and glucose 11.1. The organs cleaned of adhering fat and connective tissue and cut about segments of 1–1.5 cm long. Isolated tissues were suspended in isolated organ baths filled with 20 ml of Krebs' solution (pH 7.4) continuously aerated with a mixture of 5% CO_2 and 95% O_2 at 37 °C. One end of the isolated organs was connected to a tissue holder and the other to isotonic transducers (Ugo Basile, No. 7006, Varese Italy) which were connected to a two channel pen recorder (Ugo Basile, No. 7070 'Gemini', Varese, Italy). The tissues were equilibrated by incubation in the Krebs' solution for 60 min under a resting tension of 1.0 g. During the incubation period, cumulative concentration-response curves were obtained with acetylcholine chloride for organs in the absence and presence of the test compounds. After a reproducible concentration-response relationship was obtained by repetition of the same procedure, test substance (0.1, 0.2 and 0.4 mL) was used for isolated fundus, duodenum and ileum (Staff of Edinburgh 1970) Carvacrol (10^{-6}, 10^{-5} and 10^{-4} M) as dissolved in DMSO was used only for isolated rat fundus experiments. The contact time of all the test materials was 5 min.

3.3. Statistical evaluation of data

Results are presented as mean ± s.e.m. and statistical significance between groups was analysed by analysis of variance followed by the Tukey's HSD multiple comparison test and results were considered as significant where p value was < 0.05.

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