Inhibition of serotonin-induced contractions of guinea-pig ileum by *Tilia europeae* L. aqueous extract

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**ABSTRACT:** An aqueous extract of dried *Tilia europeae* L. showed a non-specific inhibitory effect on serotonin-induced contractions and no effect on histamine, acetylcholine or potassium-induced contractions of guinea-pig ileum. Maximum responses to serotonin were reduced increasingly with increasing doses of the tilia extract. Affinity of the gut to the serotonin was changed by the extract but maximum responses and affinity of the gut to the other agonists and to the potassium were not changed. Any effect of the aqueous extract of tilia was detected on the noradrenaline dose-response curves in guinea-pig aorta rings. The inhibitory effect on serotonin-induced contractions may be responsible for the effect of *Tilia europeae* as spasmolitic.

**KEYWORDS:** *Tilia europeae* L., Guinea-pig ileum, Serotonergic receptors.

**Introduction**

The aqueous extract obtained from *Tilia europeae* has been used in popular medicine as sedative/anxiolytic and spasmolitic (Martindale, 1993), but its mechanism of action is not known. In previous studies we evaluated the possible anxiolytic/sedative properties of this extract in mice by using different behavioural models. This extract shows an inhibitory effect under the [3H-GABA] uptake and a stimulation of Ca²⁺-independent release of [3H-GABA] previously accumulated in synaptosomal fraction of cerebral cortices of rats. Although the aqueous extract of tilia contains several amino acids including GABA which can explain the results obtained in vitro (Cavadas et al., 1997) but can not be directly extrapolated to possible in vivo effects of the tilia extract as tranquilizer or mild sedative because GABA does not significantly cross the blood-brain barrier (Benassi et al., 1992).

As progress in several spheres has unfolded, the envolvement of serotonergic system has expanded greatly. Studies that have been generated by the introduction of buspirone have broadened the understanding of the neurobiological bases of anxiety and depression, and have also yielded a more complete analysis of the behavioral indices of 5-HT mediated activity (Lucki, 1990; Lucki and Wieland, 1990; Wilkinson and Dourish, 1990). Progress in the area of 5-HT receptor and molecular pharmacology also has been extensive (Hamon et al., 1990; Harting et al., 1990; Lucki, 1990). In animal experiments evidence has been obtained indicating possible therapeutic use of 5-HT₃ receptor antagonists in a variety of indications including anxiety (Jones et al., 1988; Costall and Naylor, 1992), psychosis (Tricklebank, 1989), impaired memory (Costall et al., 1989), emesis and intestinal motility (Sanger, 1992) and inflammatory pain (Giordano and Rogers, 1989).

This work aimed to study the influence of *Tilia europeae* extracts on the guinea-pig ileum contractions induced by histamine, acetylcholine, potassium and serotonin comparing the effects mediated by serotonin that in presence of atropine, ketanserin and a 5-HT₃ antagonist - ondansetron (Butler et al., 1988; Costall and Naylor, 1992). We also studied the influence of this extract on the noradrenaline-induced contractions on the guinea-pig aorta.

**Materials and Methods**

**Extract preparation**

*Tilia europeae* L. inflorescences were obtained at the Botanic Garden of Coimbra and identified by the Botanic Institute of the University of Coimbra. The inflorescences were dried at room temperature and then kept at -20°C. To prepare the aqueous extract, the dried material was crushed in a mechanical mill and passed through a 20 mesh sieve. The resulting powder (3 g) was resuspended in 100 ml of boiled distilled water; after cooling, the infusion was filtered under vacuum. The contents of hydrocinnamic acids (caffeic acid as standard) and flavonoids (rutine as standard) were determined as described by Lamaison et al. (1991) and Lamaison and Carnat (1990), respectively. The extracts used in the experiments contained 0.84 ± 0.05 mg of...
caffeic acid per ml and 0.11 ± 0.03 mg of rutine per ml of extract.

All experiments were conducted in accordance with guidelines established by the European Animal Care Committee.

**Drugs**

5-hydroxytryptamine creatinine sulphate, acetylcholine chloride, histamine dihydrochloride, noradrenaline and potassium were obtained from Sigma Chemical Co., USA.

**Organ preparation and experimental procedure**

Guinea-pigs, 300-500 g, of either sex were fasted overnight, killed by stunning and bleeding and a 20 cm portion of ileum, approximately 10 cm proximal to the ileocecal junction and the aorta were removed. The descending aorta was freed of connective tissues and cut into 2-3 mm rings. The ileum was cut into 1-2 cm segments and the lumen rinsed by gentle flushing with Tyrode buffer. The aorta and longitudinal muscles were suspended in 5-ml tissue baths containing Krebs and Tyrode solutions, respectively, maintained at 37º C with oxygenation (95% O₂-5% CO₂) and were allowed to equilibrate for 30 min. The buffer in the tissue bath was replaced at least every 15 min during the duration of the experiments. Tension (1 g) was placed on the tissues and they were allowed to equilibrate for an additional hour. All responses were recorded isometrically using force-displacement transducers and a pen recorder.

After the initial concentration-response curves with potassium, histamine, acetylcholine or 5-hydroxytryptamine (5-HT) (6 nM-0.3 mM) on the ileum and noradrenaline (NA, 6 nM-130 µM) on the aorta the tissues were allowed 1h to recover before a second curve was generated; after several washings, tilia extract (0.2 to 1.8 mg/ml) or the solvent were added and another curve performed. Some of the experiments with 5-HT were performed in the presence of 0.1 µM ketanserin, ketanserin plus 0.07 µM atropine and others in the presence of ketanserin plus ondansetr on (0.1 µM). Tissues were then incubated with this drugs for 15 min and then remained in the baths during the generation of the dose-response curve.

**Data handling and statistics**

Values are expressed as mean ± s.e.m. of at least three separate experiments. Drug potency was expressed as the concentration eliciting 50% of the maximal response (EC50). This values were calculated as the amount of compound required to reduce the response to agonist to 50% of the pre-dose control in the same animal.

Differences between dose-response curves were evaluated by ANOVA analysis to determine statistical significance; unless otherwise indicated P < 0.05 was considered statistically significant.

**Results**

Effect of tilia extract on agonist-induced dose-response curves on ileum.

The effects of tilia extract was examined for direct responses on the guinea-pig isolated ileum. Acetylcholine or histamine-induced contractions were not significantly affected by tilia extract (0.2-1.8 mg/ml) (Fig. 1 and Fig. 2);

![Figure 1. Acetylcholine dose-response curves in guinea-pig ileum. Comparative study in the presence of Tilia europeae. Results are expressed as a percent of the tissue response to 1 µM acetylcholine (mean ± sem., n=12).](image1)

![Figure 2. The effects of tilia extract on histamine-induced contractions in guinea-pig ileum (1 µM histamine). (mean ± sem., n=12).](image2)

5-HT elicited dose-dependent contractions of the ileum. A series of pharmacological experiments was performed to characterize this contraction. Consecutive cumulative concentration-response curves were developed for 5-HT based on a 1-min drug exposure and 1h recovery period between curves. Thus, experiments which examined the effect of ligands on the response to 5-HT were performed and then comparing ligand-treated tissues with appropriately matched controls in the second concentration-response curve. Ketanserin decreased the maximal response to 5-HT,
indicating that this contraction is mediated also by 5-HT₂ receptors. Ketanserine plus tilia extract decrease more the maximal response to 5-HT (51.1±12 %) and ketanserin plus tilia plus atropine (5-HT₁ receptors) abolished that response (Fig. 3).

In guinea-pig ileum 5-HT was non-competitively antagonized, in a concentration-dependent way, by tilia extracts, the maximal effect obtained in the presence of 0.2, 0.6 or 1.8 mg/ml tilia was 80 ± 10.5, 52.5 ± 15.3 or 37.3 ± 10.4 %, respectively (Fig. 4).

While multiple 5-hydroxytryptamine receptors have been identified in the guinea-pig ileum, the 5-HT₂ receptors have been most intimately associated with the prokinetic effects of the drugs. So, we sought to determine if this receptor may be involved in the contractions induced by 5-HT. The initial method was an attempt to block 5-HT response with tilia extract in presence of ketanserin. The second approach for characterizing potential 5-HT₃ receptor interactions utilized selective antagonist (ondansetron) in the same experimental conditions. There was a synergistic effect between tilia and ondansetron (Fig. 5).

**DISCUSSION**

Clinical studies support the view of an involvement of 5-HT in the regulation of gastrointestinal motility: 5-HT₃ antagonists have been shown to possess gastrokinetic and anti-emetic properties (Libundgust and Lancranjan, 1987).

In guinea-pig ileum, serotonin is known to exert two effects, a primary action on neuronal receptors to effect release of acetylcholine and a minor action on smooth muscle receptors (Rocha e Silva et al., 1953; Day and Vane, 1963; Costa and Furness, 1979). The relative contribution of the direct and indirect actions of serotonin on the contractile response in the guinea-pig ileum varies with the segment of the gastrointestinal tract used (Costa e Furness, 1979). In most vascular tissues as well as in the rat uterus and guinea-pig trachea contractions to serotonin are mediated by its interaction with 5-HT₂ receptors. In gastrointestinal smooth muscle, responses to serotonin are clearly not mediated by interaction with 5-HT₂ receptors. The exact nature of the receptors responsible for the contractile response to serotonin in both the stomach fundus and guinea-pig ileum have not yet been established. The results obtained by Cohen et al. (1985) and Fox & Morton (1990) showed that there are at least three different serotonin receptors and 5-HT₃ receptor activation results in acetylcholine release from enteric nerves studied primarily in guinea-pig ileum. Such responses to serotonin-induced activation of enteric nerves are sensitive to atropine and tetradotoxin. In our preparation tilia extract did not changed the maximal response to acetycholine neither histamine. Thus the effect of tilia extracts on the guinea-pig ileum is nonco-linerigic and nonhistaminergic mechanism. Also, tilia extract did not change the maximal response to nor-
Tilia extract shows a potent antagonist action of 5-HT contractions and the results analysis was consistent with a non-competitive antagonist mechanism. Once the maximum responses to the agonist serotonin were dose dependent and significantly reduced, we conclude that the inhibitory effect is not competitive, at the receptor level (Schild, 1954). This drug causes non-parallel rightward displacement of concentration-response curve with a decrease of the maximal effect. The depression in maximum response to agonist could be explained by a hemiequilibrium state (Kenakin, 1987) that, due to slow antagonist dissociation, agonist may only equilibrate with a reduced receptor population. Other explanation could include an additional action of the antagonists at a site beyond the receptor, for instance a direct block of the cation channels which mediate the Na+ fluxes carrying 5-HT3-induced depolarization (Rhodes et al., 1993).

Non-competitive antagonism of the extract in this preparation may be comparable to obtained with the 5-HT3 antagonists, ICS 205930, MDL 72222 and quipazine, previously reported (Ireland and Tyers, 1987).

The 5-HT3-related emesis presumably originates from a central effect involving the area postrema (Higgins et al., 1989) but it may also partly involve a peripheral effect. Recently, ondansetron has been proposed as a safe and effective antiemetic for the treatment of postoperative nausea and vomiting after general anesthesia (Larijani et al., 1991).

The 5-HT3 antagonists are not active in a morphine-induced emesis (Miner et al., 1987) and are inefficient in "motion-sickness" in cats (Lucot, 1989) or in humans (Stott et al., 1989); these results suggest that emesis may be provoked by several distinct mechanisms.

A great deal of interest is developing in the potential activities of the 5-HT3 receptors in psychiatry, in particular they may play a role in the re-emergence of the neurochemical processes involved in anxiety. Indeed, low doses of ondansetron, granisetron, tropisetron, MDL 72222 and zacopride increase social interaction in rats under averse conditions (Jones et al., 1988; Young and Johnson, 1991).

Anxiolytic properties are not evidenced in all experimental tests, moreover, ondansetron and granisetron at high doses exhibit anxiogenic properties (Costall et al., 1988 a). These results and the fact that 5-HT3 antagonists have a delay of onset of action longer than that of the benzodiazepines indicate that these drugs operate by different modes of action.

Interesting properties have been revealed by tilia extract in experimental models of behaviour. For example, tilia, unlike diazepam showed anxiolytic properties on the elevated plus maze, hole board, chimney, and object-burying tests (Cotrim et al., 1997).

The present experiments demonstrate that tilia extract is an antagonist at peripheral 5-HT3 receptors that associate to the results obtained in vivo suggests that action on central nervous system mediated by this sub-type of serotonergic receptors. More studies are needed namely that related with the pharmacological action on the central nervous system to confirm this hypothesis.