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ORIGINAL ARTICLE

Inhibitory effect of the herbal antidepressant St. John's wort (*Hypericum perforatum*) on rat gastric motility

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Abstract St. John's wort (*Hypericum perforatum*) is a highly popular and effective herbal antidepressant that clinically interacts with a number of conventional drugs. Because alterations in gastric emptying can cause pharmacokinetic interactions, in the present study we evaluated the effect of a standardized extract prepared from the flowering tops of *Hypericum perforatum* (SJW extract) on rat gastric motility. Orally administered SJW extract delayed gastric emptying in vivo. In vitro studies showed that SJW extract was significantly more active in inhibiting acetylcholine (or prostaglandin E_2)-induced contractions than electrical field stimulation (EFS)-induced contractions. The effect of SJW extract on EFS-induced contractions was unaffected by drugs that inhibit intrinsic inhibitory nerves or by tachykinin antagonists, but it was reduced by the 5-hydroxytryptamine antagonist methysergide. The inhibitory effect of SJW extract on acetylcholine-induced contractions was reduced by the sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor cyclopiazonic acid, but not by the L-type Ca^{2+} channel blocker nifedipine or by methysergide. Among the chemical constituents of SJW extract tested, hyperforin and, to a lesser extent, the flavonoids kaempferol and quercitrin, inhibited acetylcholine-induced contractions. It is concluded that SJW has a direct inhibitory effect on smooth muscle and could also possibly modulate gastric neurotransmission. If extended to humans, the inhibitory effect of SJW extract on gastric emptying in vivo could contribute, at least in part, to the clinical pharmacokinetic interactions between conventional medicines and this herbal antidepressant.

Keywords Gastric emptying · Flavonoids · *Hypericum perforatum* · Herb-drug interaction · Hyperforin · Gastric neurotransmision

Abbreviations

SJW extract	standardized extract prepared from the flowering tops of *Hypericum perforatum*
EFS	electrical field stimulation
L-NAME	N^G-nitro-L-arginine methyl ester
DMSO	dimethyl sulphoxide
hCGRP	VIP antagonist [Ac-Tyr^1,D-Phe^2]GRF 1–29 amide
1–29	
Prostaglandin E_2	PGE_2

Introduction

St. John's wort was among the top 10 herbs sold in the USA in the 2005, where sales in the food, drug, and mass market channel were estimated at nearly $10 million (Blumenthal et al. 2006). Common throughout Europe and the USA, St. John's wort (*Hypericum perforatum* L., Family Clusiaceae; SJW) is an erect perennial herb with yellow flowers that has been used as a medicinal herb since ancient times. Today, SJW is used in many countries mainly for the treatment of depressive disorders. The efficacy of SJW standardized extract in treating mild to moderate depressive disorders has been demonstrated in numerous double-blind, placebo-controlled randomized trials and confirmed by many meta-analyses (Di Carlo et al. 2001;

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De Smet 2002; Linde et al. 2005). Numerous comparative trials indicated that it may be as effective as conventional antidepressants (Szegedi et al. 2005) and appears to offer significant advantages over conventional antidepressants because it is associated with fewer adverse reactions (De Smet 2002; Knuppel and Linde 2004). However, case reports and clinical studies have highlighted the possibility of relevant—and in some cases life-threatening—pharmacological interactions with prescribed drugs (Ernst 1999; Mannel 2004; Izzo 2005; Williamson 2005). St. John's wort has been shown to lower plasma concentration (as well as the pharmacological effect) of a number of synthetic drugs, including drugs with a narrow therapeutic index (Mannel 2004; Izzo 2005). Notably, cases of organ rejection or unplanned pregnancy have been reported when St. John's wort was used concomitantly with cyclosporine or oral contraceptives, respectively (Izzo 2005; Hu et al. 2005). Induction of P-glycoprotein (which can reduce the intestinal absorption and increase the renal elimination of drugs) and cytochrome enzymes (with an increase in drug metabolism) by St. John's wort have been proposed as possible mechanisms to explain such pharmacokinetic interactions (Zhou et al. 2004; Whitten et al. 2006).

It is well known that drugs influencing gastric emptying can have a significant effect on drug uptake. Indeed, a delay in absorption could result as a consequence of delayed gastric emptying (Lipka et al. 1996). Because St. John's wort has been shown to decrease the absorption of a number of conventional drugs, in the present study we evaluated the effect of a standardized extract prepared from the flowering top of *Hypericum perforatum* (SJW extract) on gastric motility in the rat in vivo. In order to elucidate the mechanism underlying the observed effect, we also performed experiments on isolated gastric tissues.

Materials and methods

Animals

Male Wistar rats (200–240 g), purchased from Harlan Italy (San Pietro al Natisone, Italy) were maintained under controlled conditions of temperature (24±2°C) and humidity (60%) until used. The rats had free access to water and food. All experiments complied with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

Isolated stomach

Rats were killed by asphyxiation with CO_2. The stomach was removed immediately and placed in Krebs solution (mM: NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, $MgSO_4$ 1.5, and glucose 11). Segments (1 cm) of longitudinal full-thickness strips were prepared from the gastric fundus. The muscle strips were suspended in an organ bath containing 20 ml Krebs equilibrated with 95% O_2, 5% CO_2 at 37°C. The strips were connected to an isotonic transducer (load 1 *g*) connected to Graphtec recording apparatus (Linearcorder, Jokohama, Japan). After a minimum 60-min equilibration period, the tissues were subjected to electrical field stimulation (EFS, 5 Hz for 1 s, 500 mA, 0.25-ms pulse duration), delivered via electrodes placed around the tissue using a digital stimulator LE12106 (Pan Lab-2 Biological Instruments, Cornella (Barcelona), Spain).

After stable control contractions evoked by EFS had been recorded (contractions were stable and reproducible for a period of at least 240 min), the responses were observed in the presence of increasing cumulative concentrations of an SJW extract (1–1000 μg ml^{-1}). The contact time for each concentration of SJW was 20 min. Preliminary experiments showed that this contact time was sufficient for SJW to achieve the maximum effect; in addition, no difference in potency was observed when SJW was added in a cumulative or in a noncumulative way.

The effect of SJW extract was also evaluated after the administration in the bath (contact time 30 min) of the NK_1 receptor antagonist SR 140333 (10^{-7} M), the NK_2 receptor antagonist SR 48968 (10^{-6} M), the NK_3 receptor antagonist SR 142821 (10^{-7} M), propranolol (10^{-6} M) + phentolamine (10^{-6} M) (to block adrenergic receptors), methysergide (10^{-6} M) (to block 5-hydroxytryptamine receptors), haloperidol (10^{-7} M) (to block dopamine receptors), or of a combination of N^G-nitro-L-arginine methyl ester (L-NAME 3×10^{-4} M), apamin (10^{-7} M) and the VIP antagonist [Ac-Tyr^1,D-Phe^2]GRF 1–29 amide (hCGRP 1–29, 1.5×10^{-6} M) (to block the gastric intrinsic innervation). The concentrations of antagonists/inhibitors used here were selected on the basis of previous work (Nocerino et al. 2002; Capasso et al. 2004, 2006). In preliminary experiments, the effect of tetrodotoxin (3×10^{-7} M) or atropine (10^{-6} M) on EFS-induced contractions was evaluated after a 10-min contact time.

In some experiments, the effect of SJW extract (1–1000 μg ml^{-1}) was also evaluated (contact time 20 min) on the contractions produced by exogenous acetylcholine (10^{-6} M). Acetylcholine was left in contact with the tissue for 30 s and then washed out. The effect of SJW extract on acetylcholine-induced contractions was also evaluated after the administration in the bath (contact time 30 min) of cyclopiazonic acid (10^{-5} M) (a sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor) and of nifedipine (10^{-6} M) (a L-type calcium channel blocker). In another set of experiments, we evaluated the effect of SJW extract (1–1000 μg ml^{-1}) on the contractions evoked by prostaglandin E_2 (PGE_2, 3×10^{-8} M).

PGE_2 was left in contact with the tissue for 2 min and then washed out. The 10^{-6} M concentration of acetylcholine and the 3×10^{-8} M concentration of PGE_2 gave a contractile response that was similar in amplitude to that of electrical stimulation.

Finally, we evaluated the effect of a number of St. John's wort ingredients, namely hyperforin (10^{-8}–10^{-4} M), hypericin (10^{-8}–10^{-4} M), rutin (10^{-8}–10^{-4} M), quercitrin (10^{-8}–10^{-4} M), and kaempferol (10^{-8}–10^{-4} M) on acetylcholine-induced contractions (contact time: 20 min for each concentration). In some experiments, the effect of hyperforin was evaluated in the presence of cyclopiazonic acid (10^{-5} M).

Gastric emptying

Gastric emptying was evaluated as previously described (El-Salhy 2001). Briefly, after an overnight fast, the animals received by gavage 1 ml/100 g of a solution of 50 mg phenol red in 100 ml 1.5% carboxymethylcellulose, which was constantly stirred and held at 37°C. After 20 min, rats were euthanized and the stomach was quickly ligated at the lower oesophageal sphincter and pyloric region and removed. The stomach was opened, and its contents were poured in a test tube and washed with 4 ml distilled water. At the end of the experiment, 2 ml NaOH (1 M) was added to each tube to develop the maximum intensity of color. The solutions were assayed with a spectrometer at 560 nm. Percent gastric emptying was calculated according to the following formula: 100× (1 - amount of phenol red recovered after 20 min/amount of phenol red recovered after 0 min). SJW extract (100–600 mg kg^{-1}) was given orally 60 min before the administration of the marker (phenol red). The SJW extract doses were selected from previous work (Tagliamonte 1999; Gambarana et al. 2001; Butterweck et al. 2001).

Drugs

SJW extract (dry hydromethanolic extract prepared from the flowering tops of *Hypericum perforatum,* standardized to contain 0.3% hypericin), hypericin, and hyperforin were a gift from Indena (Milan, Italy). Acetylcholine hydrochloride, N^G-nitro-L-arginine methyl ester (L-NAME), apamin, phentolamine hydrochloride, propranolol hydrochloride, methysergide maleate, haloperidol, tetrodotoxin, atropine sulfate, prostaglandin E_2, cyclopiazonic acid, quercitrin, rutin, and kaempferol were purchased from Sigma (Milan, Italy). The VIP antagonist [Ac-Tyr^1,D-Phe^2]GRF 1–29 amide (human; hGRF 1–29) was purchased from Tocris Cookson (Bristol, UK). A physiological salt solution (Krebs) gassed with 95% O_2 and 5% CO_2 of the following composition (mM) was used: NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.5, $CaCl_2$ 2.5 and glucose 11. SR 140333 (*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl-]ethyl}-4-phenyl-1-axoniabicyclo[2,2,2,]octane chloride, SR 48968 (*S*)-*N*-methyl-*N*[4-(4-acetylamono-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide hydrochloride, and SR 142801 (*S*)-(*N*)-(1-3-(1-benzoyl-3-(3,4-dichlorophenil)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide were a gift from SANO FI-*Recherche* (Montpellier, France).

Haloperidol, hypericin, hyperforin, quercitrin, rutin, and kaempferol were dissolved in dimethyl sulphoxide (DMSO). The other drugs, including SJW extract, were dissolved in distilled water (SJW extract was dissolved in water for in vivo experiments). PGE_2 was first dissolved in ethanol (10^{-2} M) and afterwards diluted in distilled water. DMSO and diluted ethanol (both less than 0.01%) did not modify EFS-induced contractions.

Statistical analysis

Data are expressed as the mean ± s.e.m. To determine statistical significance, Student's *t*-test was used for comparing a single treatment mean with a control mean, and a one-way analysis of variance followed by a Tukey-Kramer multiple comparisons test was used for analysis of multiple treatment means. Analysis of variance (two-way) was used to compare different cumulative concentration-effect curves. A *P*-value less than 0.05 was considered significant.

Results

Gastric emptying

Oral administration of SJW extract (100–600 mg kg^{-1}) produced a dose-dependent decrease in gastric emptying (Fig. 1). A significant inhibitory effect was achieved for the 600 mg/kg dose.

Electrical field stimulation (EFS)-induced contractions

EFS of the rat stomach evoked contractions that were abolished by tetrodotoxin (3×10^{-7} M) or atropine (10^{-6} M), thus indicating that these contractions were due to the release of acetylcholine from enteric nerves. In addition, these contractions were not significantly modified by SR 140333, SR 48968, or SR 142821 (antagonists of NK_1, NK_2, and NK_3 receptors, respectively), phentolamine (10^{-6} M) plus propranolol (10^{-6} M), haloperidol (10^{-7} M) (% variation: SR 140333 3±3%, SR 48968 4±3%, SR 142821 5±4%, phentolamine plus propranolol 2±3%, haloperidol 20±5%). Methysergide or a combination of

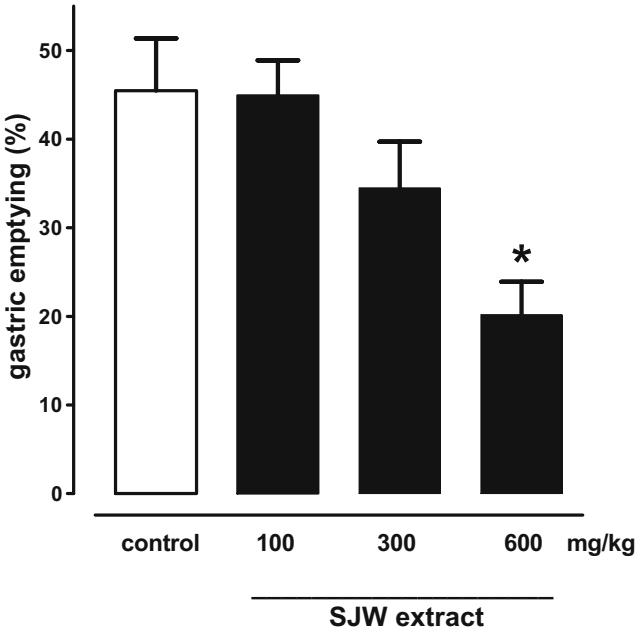

Fig. 1 Inhibitory effect of a standardized extract prepared from the flowering tops of *Hypericum perforatum* (SJW extract; 100–600 mg kg^{-1}, per os) on gastric emptying in the rat in vivo. Results are mean±s.e.m. of 10–12 animals for each experimental group. *$P<0.05$ vs. control

L-NAME (3×10^{-4} M), apamin (10^{-7} M), and the VIP antagonist hGRF 1–29 (1.5×10^{-6} M) significantly increased EFS-induced contractions (methysergide 38±4%, $P<0.05$, $n=7$–8; L-NAME plus apamin plus VIP antagonist 183±15%, $P<0.001$, $n=7$–8).

SJW extract (1–1000 μg ml^{-1}) decreased, in a concentration-dependent manner, the amplitude of EFS-evoked contractions (Fig. 2). The inhibitory effect of SJW extract on EFS-induced contractions was unaffected by tachykinin NK_1, NK_2, and NK_3 antagonists, or by a combination of drugs that block the intrinsic inhibitory innervation of the rat stomach (i.e., a combination of L-NAME 3×10^{-4} M, apamin 10^{-7} M plus the VIP antagonist hGRF 1–29 1.5×10^{-6} M) (data not shown). However, treatment of the tissue with the 5-hydroxytryptamine antagonist methysergide (10^{-6} M), but not haloperidol (10^{-7} M, a dopamine antagonist) or a combination of drugs that block α- and β-adrenergic receptors (i.e., phentolamine 10^{-6} M plus propranolol 10^{-6} M) reduced the inhibitory response of SJW extract on EFS-evoked contractions (Fig. 3).

Acetylcholine- and PGE_2-induced contractions

SJW extract (1—1000 μg ml^{-1}) decreased, in a concentration-dependent manner, the acetylcholine- and PGE_2-induced contractions (Fig. 2). SJW extract was significantly more active in inhibiting the contractions induced by agonists (i.e., acetylcholine and PGE_2) than the contractions induced by EFS (Fig. 2). The inhibitory effect of SJW extract on acetylcholine-induced (10^{-6} M) contractions was significantly reduced by cyclopiazonic acid (10^{-5} M), a compound that induces a net loss of calcium from the sarcoplasmic reticulum, but not by the L-type Ca^{2+} channel blocker nifedipine (10^{-6} M) (Fig. 4) or by the 5-hydroxytryptamine receptor antagonist methysergide (10^{-6} M, data not shown).

Figure 5 shows the effect of a number of St. John's wort constituents on acetylcholine-induced contractions. Among the chemical compounds tested, hyperforin and, to a lesser extent, the flavonoids kaempferol and quercitrin reduced acetylcholine-induced contractions. Hypericin and the flavonoid rutin were without significant effect (Fig. 5). The inhibitory effect of hyperforin on acetylcholine-induced contractions was significantly reduced by cyclopiazonic acid (Fig. 6). In the absence of SJW extract, nifedipine and cyclopiazonic acid significantly reduced acetylcholine-induced contractions (nifedipine: 59±4% inhibition, $P<0.01$; cyclopiazonic acid: 38.2±5%, $P<0.05$, $n=7$–8). However, cyclopiazonic acid, given alone, caused an increase in the basal tone of the gastric fundus.

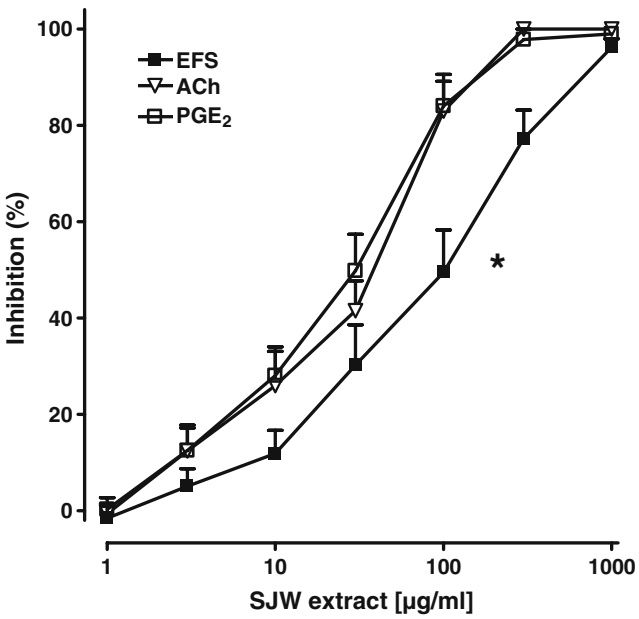

Fig. 2 Inhibitory effect of a standardized extract prepared from the flowering tops of *Hypericum perforatum* (SJW extract, 1–1000 μg ml^{-1}) on the contractile response produced by electrical field stimulation (EFS), acetylcholine (ACh, 10^{-6} M), or PGE_2 (3×10^{-8} M) in the isolated rat stomach. The *ordinates* show the percentage of inhibition of the control response. *Vertical lines* show s.e.m. Each *point* represents the mean of 7–8 animals. *$P<0.05$ vs. ACh (or PGE_2) [i.e., the curve representing the inhibitory effect of SJW extract on EFS-induced contractions was statistically different from the curve representing the inhibitory effect of SJW extract on ACh- (or PGE_2)-induced contractions]

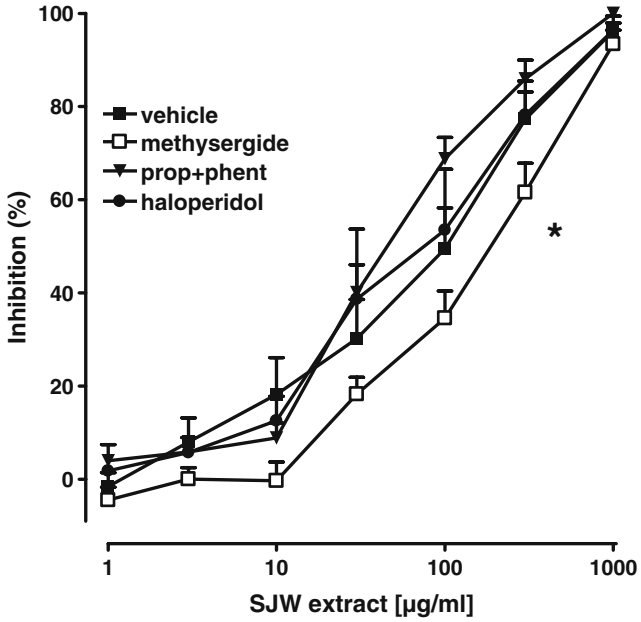

Fig. 3 Electrical field stimulation (EFS)-induced contractions in the isolated rat stomach: inhibitory effect of a standardized extract prepared from the flowering tops of *Hypericum perforatum* (SJW extract 1–1000 µg ml^{-1}) alone (vehicle) or in presence of methysergide (10^{-6} M) (to block 5-hydroxytryptamine receptors), haloperidol (10^{-7} M) (to block dopamine receptors), or a combination of propranolol (10^{-6} M) and phentolamine (10^{-6} M) (prop + phent, to block α- and β-adrenergic receptors). The *ordinates* show the percentage of inhibition of the control response. *Vertical lines* show s.e.m. Each *point* represents the mean of 7–8 animals. **P*<0.01 vs. vehicle (significance among the methysergide and the vehicle curves)

In the absence of any stimulus, SJW extract did not affect basal gastric tension (data not shown).

Discussion

In the present study, we have shown that a standardized extract obtained from the flowering tops of St. John's wort delayed gastric emptying. These results not only broaden the pharmacological spectrum of action of this herb, but also suggest a possible new mechanism to explain pharmacokinetic interactions between St. John's wort and other drugs.

There are few reports on the effect of SJW on gastrointestinal functions. Previous investigators have shown that extracts from *Hypericum perforatum* decreased gastric acid secretion in pylorus-ligated rats (Abdel-Salam 2005), reduced spontaneous contractions of the isolated rabbit jejunum (Gilani et al. 2005), inhibited irinotecan-induced diarrhea and intestinal damage in rats (Hu et al. 2006), and inhibited iNOS expression in human intestinal cell lines (Tedeschi et al. 2003). In the present study, we have shown that SJW extract delayed gastric emptying in a dose-dependent fashion. SJW extract reduced gastric motility at a dose-range previously shown to be effective in experimental models that are predictive of antidepressant activity (Tagliamonte 1999; Gambarana et al. 2001; Butterweck et al. 2001; Di Carlo et al. 2001). Although gastric emptying involves the fundus, corpus, antrum, pylorus, and, importantly, antroduodenal contractility (Koch and Stern 1996; Read and Houghton 1989), in the present manuscript, in order to yield insights into the mechanism(s) underlying the effect on gastric emptying here observed, we performed experiments on the isolated rat stomach fundus. We found that SJW extract reduced, in a concentration-dependent manner, acetylcholine-, PGE_2- and EFS-stimulated contractions in the isolated stomach. Moreover, SJW extract preferentially inhibited the contractions induced by agonists (i.e., acetylcholine and PGE_2) rather than the contractions elicited by EFS. The different potency of the effect of SJW extract on the contractions evoked by EFS (which are mediated by the release of acetylcholine from nerves) and by agonists (which directly contract smooth muscles) could indicate that this herb extract has an effect not limited to smooth muscle.

Biochemical and animal studies suggest that the antidepressant St. John's wort inhibits the synaptosomal uptake of a number of neurotransmitters including dopamine, noradrenaline, and 5-hydroxytryptamine. Moreover, in vitro receptor screening of pure constituents of St. John's wort

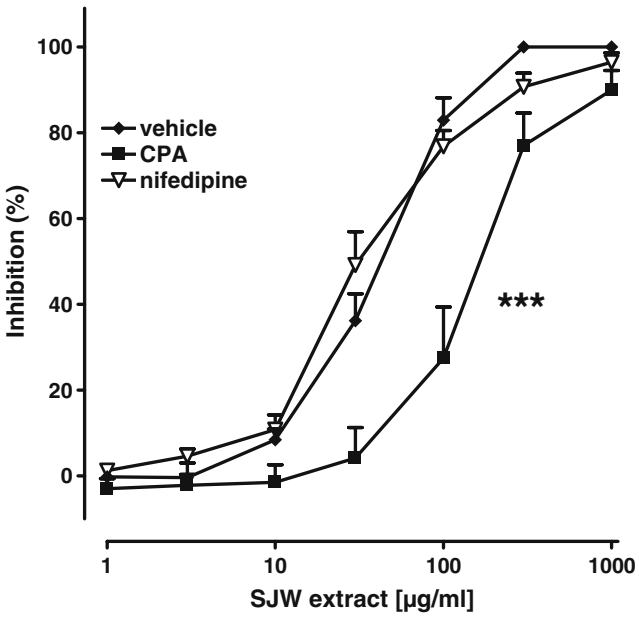

Fig. 4 Acetylcholine-induced (10^{-6} M) contractions in the isolated rat stomach. Inhibitory effect of a standardized extract prepared from the flowering tops of *Hypericum perforatum* alone (vehicle), or in the presence of cyclopiazonic acid (CPA, 10^{-5} M) (a sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor) or of nifedipine (10^{-6} M) (a L-type calcium channel blocker). The *ordinates* show the percentage of inhibition of control response. *Vertical lines* show s.e.m. Each *point* represents the mean of 7–8 animals. ****P*<0.01 vs. vehicle (significance among the CPA and the vehicle curves)

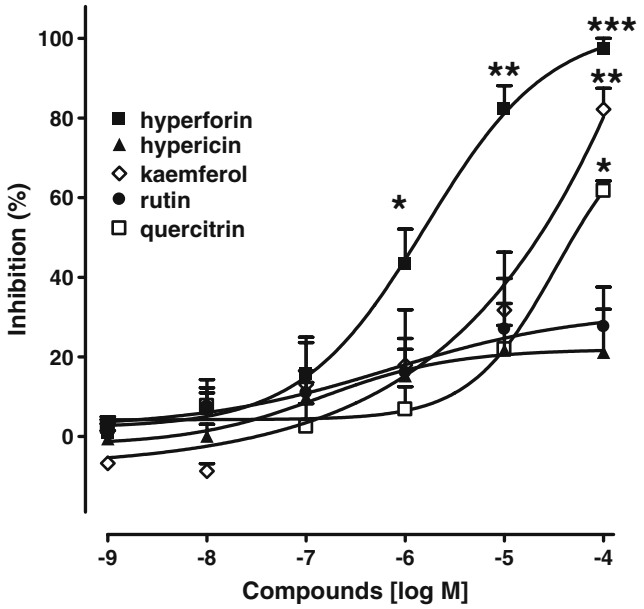

Fig. 5 Acetylcholine-induced (10^{-6} M) contractions in the isolated rat stomach: effect of some chemical compounds of *Hypericum perforatum* (hyperforin, hypericin, and the flavonoids kaempferol, rutin, and quercitrin). The *ordinates* show the percentage of inhibition of the control response. *Vertical lines* show s.e.m. Each *point* represents the mean of 6–8 animals. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ vs. corresponding control

revealed interactions with serotonin (5-HT_{1D}, 5-HT_{2c}), dopamine (D_3 and D_4), and β-adrenergic receptors (Butterweck et al. 2002; Simbrey et al. 2004). Therefore we evaluated the effect of SJW extract in the presence of methysergide (a 5-hydroxytryptamine receptor antagonist), haloperidol (a dopamine receptor antagonist), and phentolamine plus propranolol (a combination of drugs that blocks the action of noradrenaline on α-adrenergic and β-adrenergic receptors). Our results revealed an involvement of 5-hydroxytryptamine receptors since the inhibitory effect of SJW on EFS-induced contractions was significantly reduced by methysergide. It is likely that the site of action of 5-hydroxytryptamine is at the prejunctional level, since methysergide did not modify the inhibitory effect of SJW extract on acetylcholine-induced contractions. The ability of 5-HT to affect gastroenteric transmission and motility via activation of a number of receptor subtypes including 5-HT_{1A}, 5-HT_{1p}, 5-HT_3, 5-HT_4, and 5-HT_7 receptors is well documented (Taniyama et al. 2000; Gershon and Tack 2007). Other evidence also suggests that St. John's wort may affect central 5-HT neurotransmission in vivo (Mennini and Gobbi 2004).

Our experiments excluded the possible involvement of gastric inhibitory nerves (experiments with L-NAME, apamin, and VIP antagonist) and the release of tachykinins (experiments with tachykinin receptor antagonists) as potential contributing factors in SJW extract-mediated changes in EFS-induced contractions. Blockade of gastric inhibitory nerves or release of excitatory transmitters (i.e., acetylcholine and tachykinins) by SJW could have explained why this herbal extract was more active in inhibiting agonist (acetylcholine and PGE_2)-induced contractions rather than EFS-induced contractions. Others have shown that hyperforin, one of the main active ingredients of St. John's wort, increased rat hippocampal and striatal acetylcholine release in vivo (Buchholzer et al. 2002; Kiewert et al. 2004). However, in the present manuscript, we did not examine directly whether SJW extract evokes the release of acetylcholine from gastric nerves.

Previous studies have reported a direct myogenic inhibitory effect by St. John's wort on rabbit jejunum (Gilani et al. 2005), guinea-pig trachea (Gilani et al. 2005), rat bladder (Capasso et al. 2004), and rat/human vas deferens (Capasso et al. 2005). It is well known that smooth muscle can be activated by membrane depolarization and action potentials. Both types of activation involve an increase in cytosolic free Ca^{2+}. Among the various mechanisms through which Ca^{2+} may play a role in regulating smooth muscle contractility, in the present study we focused on the possibility that the inhibitory effect of SJW extract on acetylcholine-induced contractions might involve the release of Ca^{2+} from intracellular stores and/or the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels (Makhlouf 1987). We have shown that cyclopiazonic acid, a potent and specific inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase in smooth muscle (Grasa et al. 2004), reduced

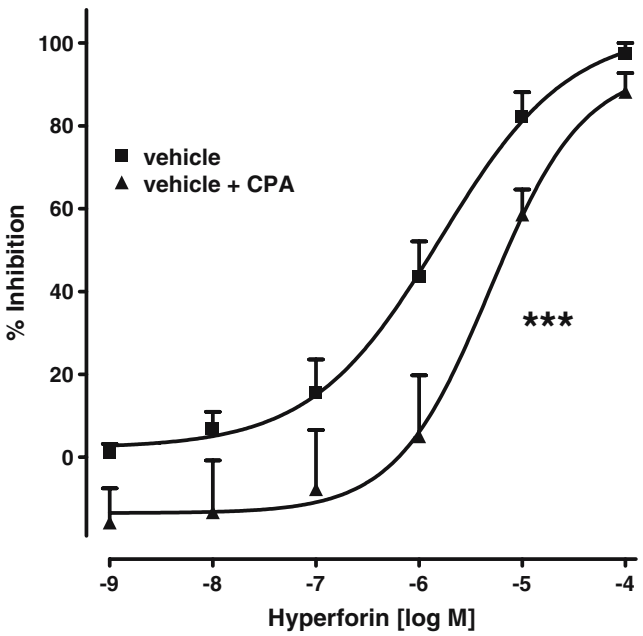

Fig. 6 Acetylcholine-induced (10^{-6} M) contractions in the isolated rat stomach: inhibitory effect of hyperforin alone (vehicle) and in the presence of cyclopiazonic acid (CPA, 10^{-5} M,) (a sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor). *Vertical lines* show s.e.m. Each *point* represents the mean of 7–8 animals. ***$P<0.01$ vs. vehicle (significance among the CPA and the vehicle curves)

the inhibitory effect of SJW extract on acetylcholine-induced contractions. The effect of cyclopiazonic acid was specific for SJW extract since we have recently shown that this Ca^{2+} blocker does not affect the inhibitory effect of another plant extract (i.e. *Boswellia serrata* extract) on acetylcholine-induced contractions (Borrelli et al. 2006). By contrast, nifedipine, at a concentration previously shown to modify the inhibitory effect of another plant extract on the gastrointestinal tract (Borrelli et al. 2006), failed to modify the inhibitory effect of SJW extract on acetylcholine-induced contractions. Collectively, such results suggest an involvement of Ca^{2+} released from sarcoplasmic stores, rather than an involvement of L-type Ca^{2+} channels, in the inhibitory effect of SJW extract. Consistent with the present results, it has been recently shown that the L-type Ca^{2+} channel antagonist verapamil did not modify the inhibitory effect of SJW extract on bladder contractility (Capasso et al. 2004). It is very unlikely that the direct inhibitory effect of SJW extract observed here is due to antimuscarinic actions, since this herbal drug also inhibited the myogenic contractions induced by PGE_2.

St. John's wort contains numerous biologically active constituents, including the naphthodianthrone hypericin, phloroglucinol derivatives (e.g., hyperforin), and flavonoids. For the treatment of depression, extracts standardized to contain 0.3% hypericin are commonly used. Such extracts may contain 2–4% flavonoids and up to 6% hyperforin (Di Carlo et al. 2001). In order to understand which of the chemical ingredients of St. John's wort is responsible for the inhibitory effect on gastric motility, we investigated the effect of pure chemical compounds. Because SJW extract delays gastric emptying in vivo and because this effect is likely exerted at the postjunctional level (see above), we investigated the effect of the pure components of St. John's wort on the contractions induced by acetylcholine only. Among the compounds tested, hyperforin (which is considered the main active ingredient of the antidepressant effect of SJW) (Di Carlo et al. 2001) and, to a lesser extent, the flavonoids kaempferol and rutin, inhibited the contractions induced by acetylcholine in the isolated rat stomach. These results suggest that multiple ingredients rather than a single chemical compound could contribute to the inhibitory effect of SJW on gastric motility, although hyperforin seems to have a major role. Consistent with the results obtained with the whole SJW extract, the inhibitory effect of hyperforin was reduced by cyclopiazonic acid, thus confirming the involvement of the sarcoplasmic Ca^{2+}. Others have shown that hyperforin, in the same range of concentrations, stimulated intracellular calcium mobilization in hamster vas deferens smooth muscle cell lines (Koch and Chatterjee 2001).

In conclusion, we have shown that a standardized extract prepared from the flowering tops of *Hypericum perforatum* reduces gastric emptying in the rat in vivo. Our studies in vitro, which aimed at investigating the mode of action of this herbal extract, revealed a possible modulatory effect on gastric neurotransmission and, most importantly, a direct inhibitory effect on smooth muscle contractility, which is likely responsible for the inhibition of gastric emptying observed in vivo. The effect of SJW extract on smooth muscle might involve the release of Ca^{2+} from sarcoplasmic stores. If extended to humans, the inhibitory effect of SJW extract on gastric emptying could constitute a novel mechanism to explain, at least in part, the clinical pharmacokinetic interactions between conventional medicines and this herbal antidepressant.

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References

Abdel-Salam OM (2005) Anti-inflammatory, antinociceptive, and gastric effects of Hypericum perforatum in rats. Sci World J 5:586–595

Blumenthal M, Ferrier KLG, Cavaliere C (2006) Total sales of herbal supplements in United States show steady growth. HerbalGram 71:64–66

Borrelli F, Capasso F, Capasso R, Ascione V, Aviello G, Longo R, Izzo AA (2006) Effect of Boswellia serrata on intestinal motility in rodents: inhibition of diarrhoea without constipation. Br J Pharmacol 148:553–560

Buchholzer ML, Dvorak C, Chatterjee SS, Klein J (2002) Dual modulation of striatal acetylcholine release by hyperforin, a constituent of St. John's wort. J Pharmacol Exp Ther 301:714–719

Butterweck V, Korte B, Winterhoff H (2001) Pharmacological and endocrine effects of Hypericum perforatum and hypericin after repeated treatment. Pharmacopsychiatry 34:S2–S7

Butterweck V, Nahrstedt A, Evans J, Hufeisen S, Rauser L, Savage J, Popadak B, Ernsberger P, Roth BL (2002) In vitro receptor screening of pure constituents of St. John's wort reveals novel interactions with a number of GPCRs. Psychopharmacology 162:193–202

Capasso R, Borrelli F, Capasso F, Mascolo N, Izzo AA (2004) Inhibitory effect of the antidepressant St. John's wort (Hypericum perforatum) on rat bladder contractility in vitro. Urology 64:168–172

Capasso R, Borrelli F, Montanaro V, Altieri V, Capasso F, Izzo AA (2005) Effects of the antidepressant St. John's wort (Hypericum perforatum) on rat and human vas deferens contractility. J Urol 173:2194–2197

Capasso R, Borrelli F, Capasso F, Siebert DJ, Stewart DJ, Zjawiony JK, Izzo AA (2006) The hallucinogenic herb Salvia divinorum and its active ingredient salvinorin A inhibit enteric cholinergic transmission in the guinea-pig ileum. Neurogastroenterol Motil 18:69–75

De Smet PA (2002) Herbal remedies. N Engl J Med 347:2046–2056

Di Carlo G, Borrelli F, Ernst E, Izzo AA (2001) St. John's wort: Prozac from the plant kingdom. Trends Pharmacol Sci 22:292–297

El-Salhy M (2001) Gastric emptying in an animal model of human diabetes type 1: relation to endocrine cells. Acta Diabetol 38:139–144

Ernst E (1999) Second thoughts about safety of St. John's wort. Lancet 354:2014–2016

Gambarana C, Ghiglieri O, Tolu P, De Montis MG, Giachetti D, Bombardelli E (2001) Pharmacological and endocrine effects of Hypericum perforatum and hypericin. Pharmacopsychiatry 34:S2–S7

Gershon MD, Tack J (2007) The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology 132:397–414

Gilani AH, Khan AU, Subhan F, Khan M (2005) Antispasmodic and bronchodilator activities of St. John's wort are putatively mediated through dual inhibition of calcium influx and phosphodiesterase. Fund Clin Pharmacol 19:695–705

Grasa L, Rebollar E, Arruebo MP, Plaza MA, Murillo MD (2004) The role of Ca2+ in the contractility of rabbit small intestine in vitro. J Physiol Pharmacol 55:639–650

Hu Z, Yang X, Ho PC, Chan SY, Heng PW, Chan E, Duan W, Koh HL, Zhou S (2005) Herb-drug interactions: a literature review. Drugs 65:1239–1282

Hu ZP, Yang XX, Chan SY, Xu AL, Duan W, Zhu YZ, Sheu FS, Boelsterli UA, Chan E, Zhang Q, Wang JC, Ee PL, Koh HL, Huang M, Zhou SF (2006) St. John's wort attenuates irinotecan-induced diarrhea via down-regulation of intestinal epithelial apoptosis. Toxicol Appl Pharmacol 216:225–237

Izzo AA (2005) Herb-drug interactions: an overview of the clinical evidence. Fund Clin Pharmacol 76:1–16

Kiewert C, Buchholzer ML, Hartmann J, Chatterjee SS, Klein J (2004) Stimulation of hippocampal acetylcholine release by hyperforin, a constituent of St. John's wort. Neurosci Lett 364:195–198

Knuppel L, Linde K (2004) Adverse effects of St. John's wort: a systematic review. J Clin Psychiatr 65:1470–1479

Koch E, Chatterjee SS (2001) Hyperforin stimulates intracellular calcium mobilisation and enhances extracellular acidification in DDT1-MF2 smooth muscle cells. Pharmacopsychiatry 34:S70–S73

Koch KL, Stern RM (1996) Functional disorders of the stomach. Semin Gastrointest Dis 7:185–195

Linde K, Berner M, Egger M, Mulrow C (2005) St. John's wort for depression: meta-analysis of randomised controlled trials. Br J Psychiatr 186:99–107

Lipka E, Crison JR, Schug BS, Blume HH, Amidon GL (1996) Drug interaction in the gastrointestinal tract and their impact on drug absorption and systemic availability: a mechanistic review. Hand Exp Pharmacol 122:13–37

Makhlouf GM (1987) Isolated smooth muscle cells of the gut. In: Jonhson LR (ed) Physiology of the gastrointestinal tract. Raven, New York, pp. 555–570

Mannel M (2004) Drug interactions with St. John's wort: mechanisms and clinical implications. Drug Saf 27:773–797

Mennini T, Gobbi M (2004) The antidepressant mechanism of Hypericum perforatum. Life Sci 75:1021–1027

Nocerino E, Izzo AA, Borrelli F, Capasso F, Capasso R, Pinto L, Sautebin L, Mascolo N (2002) Relaxant effect of capsazepine in the isolated rat ileum. Naunyn-Schmiedebergs Arch Pharmacol 365:187–192

Read NW, Houghton LA (1989) Physiology of gastric emptying and pathophysiology of gastroparesis. Gastroenterol Clin North Am 18:359–373

Simbrey K, Winterhoff H, Butterweck V (2004) Extracts of St. John's wort and various constituents affect beta-adrenergic binding in rat frontal cortex. Life Sci 74:1027–1038

Szegedi A, Kohnen R, Dienel A, Kieser M (2005) Acute treatment of moderate to severe depression with hypericum extract WS 5570 (St. John's wort): randomised controlled double blind non-inferiority trial versus paroxetine. BMJ 330:503

Tagliamonte A (1999) Efficacy of an Hypericum perforatum (St. John's wort) extract in preventing and reverting a condition of escape deficit in rats. Neuropsychopharmacology 21:247–257

Taniyama K, Makimoto N, Furuichi A, Sakurai-Yamashita Y, Nagase Y, Kaibara M, Kanematsu T (2000) Functions of peripheral 5-ydroxytryptamine receptors, especially 5-hydroxytryptamine4 receptor, in gastrointestinal motility. J Gastroenterol 35:575–582

Tedeschi E, Menegazzi M, Margotto D, Suzuki H, Forstermann U, Kleinert H (2003) Anti-inflammatory actions of St. John's wort: inhibition of human inducible nitric-oxide synthase expression by down-regulating signal transducer and activator of transcription-1alpha (STAT-1alpha) activation. J Pharmacol Exp Ther 307: 254–261

Whitten DL, Myers SP, Hawrelak JA, Wohlmuth H (2006) The effect of St. John's wort extracts on CYP3A: a systematic review of prospective clinical trials. Br J Clin Pharmacol 62:512–526

Williamson EM (2005) Interactions between herbal and conventional medicines. Expert Opin Drug Saf 4:355–378

Zhou S, Chan E, Pan SQ, Huang M, Lee EJ (2004) Pharmacokinetic interactions of drugs with St. John's wort. J Psychopharmacol 18:262–276