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Phytomedicine 13 (2006) SV 45–50
Phytomedicine
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Antioxidative properties of the gastrointestinal phytopharmaceutical remedy STW 5 (Iberogast®)

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Abstract

Since inflammation is a common mechanism of many gastrointestinal diseases, reactive oxygen metabolites may play an important role in their pathophysiology. Therefore it is interesting to know, whether phytopharmaceuticals known to modulate gastroinstinal motor function reveal also antioxdiative properties. We tested STW 5 (Iberogast®), its constituent nine different plant extracts, and some isolated compounds which are present in STW 5 for characterizing their antioxidative and radical quenching activities. The test assays consisted in pure chemical and complex celluar systems in which different types of reactive species were produced. Quantification of the effects was based on chemiluminescence reactions. The results show that all extracts contribute to the effect of the complete remedy STW 5, in the chemical systems in a strongly additve manner, in the cellular systems in a supraadditve manner. The largest contributions resulted from the extracts from peppermint and melissa leafs. Comparison of effects from isolated phytochemical compounds from the extracts with that of the extracts itself shows that usually the extract is more effective than the monosubstance which indicates also the synergism within the whole plant extracts. This means that the plant extracts present in STW 5 provide strong radical quenching activities that could also be involved in the therapeutic gastrointestinal actions.

Keywords: Antiinflammation; Reactive oxygen metabolites; Gastrointestinal diseases; Functional dyspepsia; Irritable bowel syndrome; Phytotherapeutics; AAPH; Azoamidinopropane reaction; Xanthine oxidase; Alveolar macrophages; STW 5; Iberogast®; *Iberis amara*; *Angelica archangelica*; *Chamomila recutita*; *Carum carvi*; *Silybum marianum*; *Melissa officinalis*; *Mentha piperita*; *Chelidonium majus*; *Glycyrrhiza glabra*

Introduction

Inflammation is a main mechanism of many intestinal and gastric diseases as e.g. inflammatory bowel disease, colitis or infections by *Helicobacter pylori*, and also contributing significantly to the etiology of functional gastrointestinal diseases (Collins et al., 2001). Therefore oxygen free radicals and other reactive oxygen metabolites (ROM) which are produced by leukocytes and gastrointestinal mucosal cells (Jouët et al., 1995; Teshima et al., 1998) can be regarded as central players in the pathophysiology of the gastrointestinal tract (Grisham, 1994; Gotz et al., 1997; Kolios et al., 1998; Kountouras et al., 2001; Simmonds et al., 1992). These

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0944-7113/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2006.03.018

reactive metabolites can effect the function of the gut in different ways from altering contractile properties to induction of ulcera (Kountouras et al., 2001; Moummi et al., 1991; Van der Vliet et al., 1989; Suzuki et al., 1996; Kountouras et al., 2000; Salim, 1993). With respect to therapeutic treatments it was found that some potent drugs are effective antioxidants which could be important for the curing effect (Hahm et al., 1997; Iinuma et al., 1998; Suzuki et al., 1995). Therefore it is interesting to look whether also phytotherapeutics, which have a high compliance in chronic intestinal diseases (Vozeh, 2003), and which are assumed to exert their therapeutic effects by modulating intestinal smooth muscle function (Heinle et al., 2006), are similarly effective as antioxidants. For this reason we tested the phytopharmaceutical STW 5 (Iberogast®), its components and some of the isolated compounds known to be present in these extracts in different in vitro systems with respect to their radical scavenging capability.

Materials and methods

Plant materials

A 100 ml STW 5 (Iberogast®) contains 15.0 ml of an ethanolic (extraction medium 50% ethanol by volume) fresh plant extract of *Iberis amara totalis* (bitter candytuft, 1:1.5–2.5) as well as ethanolic (extraction medium 30% ethanol by volume) plant drug extracts from: Angelica root (1:2.5–3.5) 10.0 ml, Chamomile flower (1:2.5–3.5) 20.0 ml, caraway fruit (1:2.5–3.5) 10.0 ml, milk thistle fruit (1:2.5–3.5) 10.0 ml, lemon balm leaf (1:2.5–3.5) 10.0 ml, peppermint leaf (1:2.5–3.5) 5.0 ml, greater celandine herb (1:2.5–3.5) 10.0 ml and liquorice root (1:2.5–3.5) 10.0 ml. The medicine contains 31% ethanol by volume.

Additionally, the following isolated compounds were tested: rosmarinic acid, kaempferol, luteolin, quercetin, and rutin, some of them also as glycosides, and as controls trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) and nordihydroguaretic acid.

Test systems to characterize antioxidative properties

AAPH reaction

The spontaneous thermic decomposition of 2-2′ amidinoazopropane dihydro-chloride (AAPH) was used to form initially carbon centered radicals, which in the presence of aerobic oxygen produce in secondary reactions also oxygen centered free radicals (Lissi et al., 1992). The radical generation was followed by luminol-enhanced chemiluminescence. Administration of substoechiometric concentrations (with respect to AAPH) of substances with free-radical scavenging properties caused a decrease of the light emission (quenching time) until its antioxidative potential was exhausted. Substances with prooxidatve properties increased the light emission (Germann, 2005). Chemiluminescence was detected in a luminometer (Berthold 9600, Wildbad, Germany) and expressed either as light intensity (photons counts per 3 s) or as integrated amount (photon counts per 10 min, or per min).

The typical assay consisted of 500 µl Tyrode solution (composition see Heinle et al., 2006), AAPH (10 mM), luminol (0.3 mM), and DMSO (0.5%). The concentrations of the different plant extracts and the different compounds are indicated within the results. The radical scavenging effects were quantified by determination of the quenching time, i.e. the suppression of chemiluminescence, prooxidative effects qualitatively by recording the increase of chemiluminescence.

Xanthine/xanthine oxidase system

The reaction between xanthine and xanthine oxidase (XOD) is widely used for the production of superoxide anions (Kuppusamy and Zweier, 1989). The typical assay contained in 600 µL sodium phosphate buffer (0.1 M, pH 7.8) 80 µM xanthine, 8.7 mU xanthine oxidase, 0.1 mM luminol together with 0.8% DMSO and the respective plant extract or compound (concentrations are given in results). The chemiluminescence (CL) was measured for 60 s and related to the chemiluminescence of the control without the herbal drugs (corresponding 100%).

Reactive species produced from lung macrophages

Alveolar macrophages are a very active source of ROM (Fels and Cohn, 1986). Therefore, we used cubic tissue samples (approx 5 mm per side) prepared from pig lung provided from a local slaughterhouse immediately after slaughtering the animals. Always the same part of the different lungs, the most proximal tip of both lobes was used. ROM production of the tissue sample incubated in 510 µl Tyrode solution, with luminol (0.11 mM,) and DMSO (1%) was directely measured in a Berthold biolumat for a total of 20 min: the first 10 min without addition (CL_{0-10}), the next 10 min (CL_{10-20}) with the addition of the respective herbal extract. The relation of CL_{10-20}/CL_{0-10} was determined and expressed in relation to the corresponding controls in which only the respective solute was added to the second phase of measurement.

Reactive species produced from isolated blood leukocytes

Leukocytes from blood, too, are able to secrete ROM, especially when these are activated to phagocytosis. Therefore, we used buffy coat cells provided from the local Institute of Transfusion Medicine. For each experiment, cells from one healthy donor (cell count approx. 2.5×10^5) were incubated with 500 µl Tyrode

solution and 0.11 mM luminol together with 1% DMSO. Chemiluminescence was measured during a period of 30 min: from 0 to 10 min without further addition, from 10 to 20 min with the addition of the herbal drugs and, finally, after the stimulation of phagocytosis by zymosan. Basal chemiluminescence was related to standard cell count (10^5 cells), the effects of the extracts on basal radical production, and on the degree of activiation by zymosan were expressed as the ratios $CL_{10–20}/CL_{0–10}$ and $CL_{20–30}/CL_{10–20}$, respectively.

Results

The antioxidative properties of STW 5 as evaluated by the different test system are shown in Figs. 1–4. The chemical systems provided by AAPH and the XOD reaction (Figs. 1 and 2) reveal that under these conditions the extracts from peppermint leafs and lemon balm leafs are the most potent ones, those from bitter candytuft and liquorice root the least potent ones. The complete drug STW 5 shows average activities, which correspond at the one hand to the mean additive effects calculated from the different single extracts: the quenching time calculated from the respective dilutions of the extracts equals 26.6 min and the measured value of the complete mixture STW 5 was 26.4 min. On the other hand, one can compare that, in the AAPH reaction, 0.2 µl/ml of STW 5 induces the same quenching time as 10 µM of trolox. In the xanthine/xanthine oxidase system, trolox has an IC_{50} of 5.75 µM (data not shown in the figures). Although the concentrations of extract components cannot be calculated in molar relations, it is obvious that potent antioxidants are present within the plant extracts.

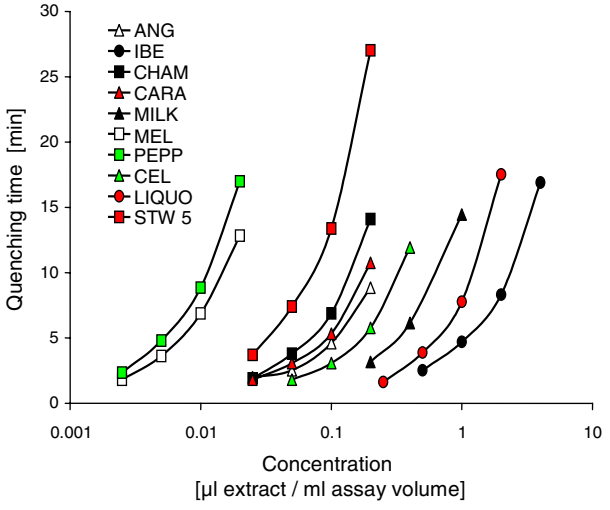

Fig. 1. Antioxidative effects in the AAPH reaction: dose dependency of quenching time of STW 5 and its constituent extracts; the symbols represent mean values of two independent measurements in each case. The correlation coefficients of the regression lines are between 0.9954 and 0.998.

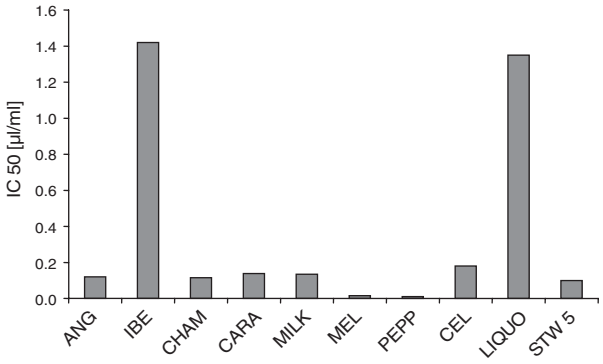

Fig. 2. Antioxidative effects in xanthine/xanthine oxidase system: The bars represent the IC_{50} values of the different extracts, i.e. the concentrations causing 50% inhibition of the control reaction without addition. These values were evaluated graphically from corresponding dose–response relations for the different extracts.

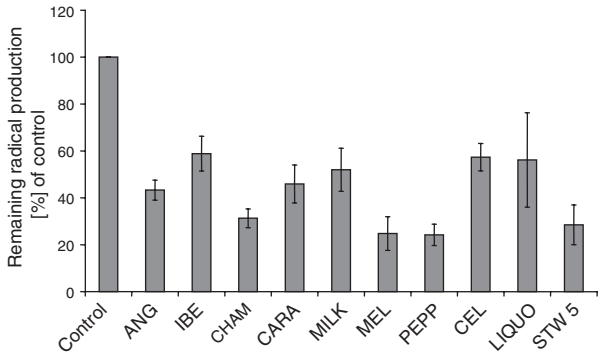

Fig. 3. Antioxidative effects in the lung model: the bars represent the remaining production of free radicals after addition of the extracts (final concentration 9.8 µl/ml assay volume) with respect to the controls. Mean values ± SD, $n = 3–4$ in each case; controls were performed during all experiments.

The application of the cellular test systems provided by lung tissue and leukocytes from blood showed also that STW 5 and its component extracts exert significant antioxidative properties (Figs. 3 and 4). In the lung model, the extracts were applied in a concentration of 9.8 µl/ml and again, peppermint and lemon balm leaves represent the most active extracts. But also bitter candytuft and liquorice root extracts were significantly active inhibiting radical production in this model. Assuming additive contributions of the different extracts to the effect of the whole mixture of STW 5, the calculated value for the remaining activity should be about 44.0%; however, the measured value corresponds to 28.5% suggesting that in this model supraadditive effects of the different extracts contribute to the total activity of STW 5. The strong antioxidant NDGA

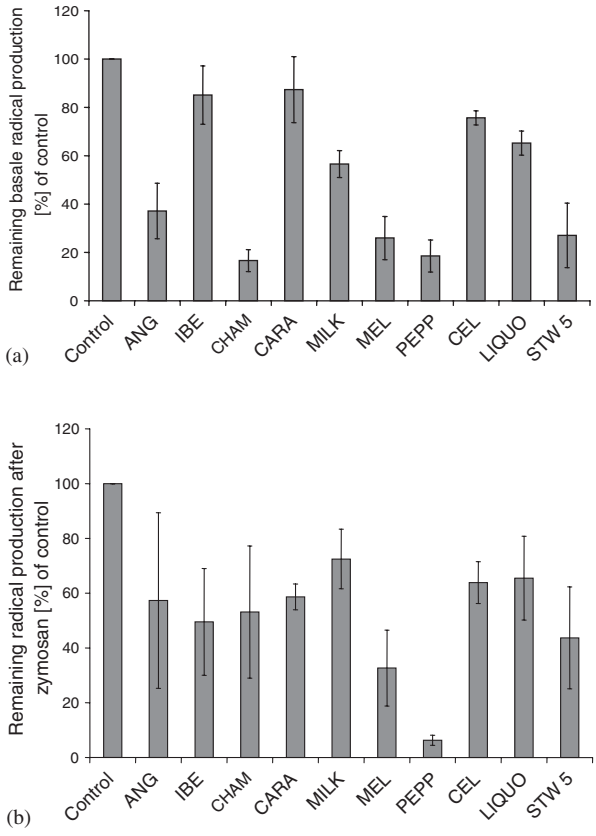

Fig. 4. Antioxidative effects in leukocytes: (a) effect on basal free-radical production. The bars represent the remaining free-radical secretion after addition of the different extracts with respect to the controls, (b) effect on degree of activation by zymosan. The bars represent the remaining degree of activation by zymosan after addition of the different extracts with respect to the controls. Final concentration of the extracts 3.3 µl/ml assay volume. Mean values ± SD, $n = 4$ in each case; controls were performed during all experiments.

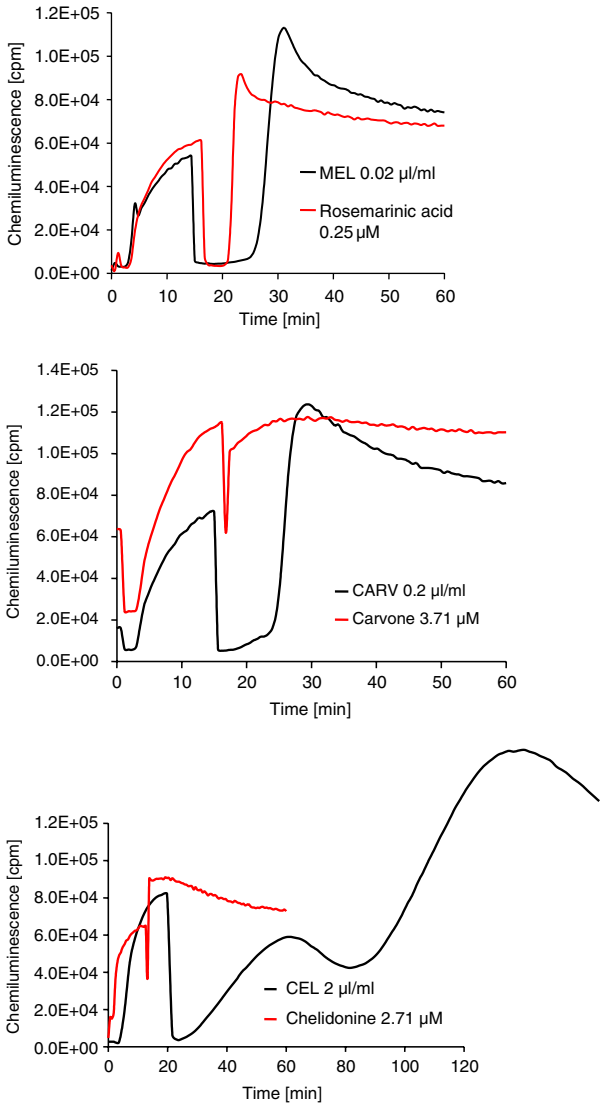

Fig. 5. Antioxidative effects in the AAPH reaction: Comparison of the effects of some extracts with that of equimolarly applied isolated components. Typical original tracings of the chemiluminescence reaction.

applied in a concentration of 25 µM caused a remaining activity of 59%.

Similar results were obtained in the leukocyte model. With a concentration of the different extracts of 3.3 µl/ml, mainly peppermint and Chamomile flower extract were active in reducing basal free-radical production, however, the zymosan-stimulated radical secretion was again most effectively inhibited by peppermint and lemon balm leafs. The latter mechanism of free-radical production is also significantly inhibited by bitter candytuft and liquirice root. Calculating again the total effects expectable from the contributions of the different extracts, the additive value of basal radical production is 52%, the measured 27%; with respect to the effect on stimulation by zymosan the calculated value is 53%, the measured one being 44%. Also these comparisons suggest that above all, the effect on basal radical production is influenced by supraadditive interactions between the different extracts, to a smaller extent also that on stimulated free-radical production. Basal and simulated free-radical production is also inhibited by trolox, 10 µM inhibits basal production to 74% and the stimulated one to 62% (data not shown in the figures).

Studies with single substances or extracts applied in the AAPH reaction are shown in Figs. 5 and 6. At first, some extracts were compared with isolated compounds applied in the same concentrations as present in the extracts. It can be shown that for all the three examples, the effect of the whole extract is different from that of the isolated compound, i.e. the extracts contain further antioxidative components (Fig. 5).

Fig. 6 shows that some flavonoids known to be present within the extracts from STW 5 reveal higher radical quenching potential than trolox. Finally, studies with different aglycons and glycosides applied in

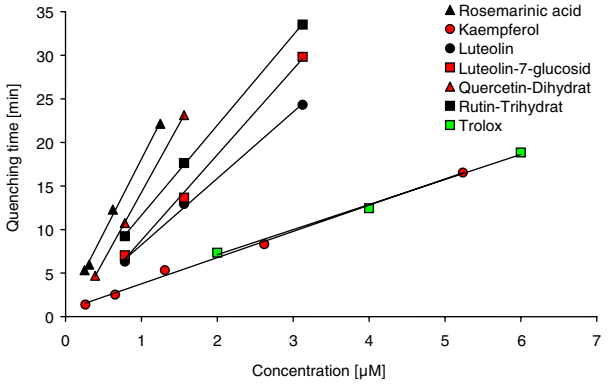

Fig. 6. Antioxidative effects in the AAPH reaction: Comparison of the dose–response reaction for the quenching time of different isolated components and trolox. The symbols represent mean values from two independant determinations.

equimolar concentrations show that in case of luteolin, the 7-glucoside is similar effective as the aglycon, whereas in case of apigenin the 7-glucoside acts as an antioxidant, the free molecule as a prooxidant. In case of kaempferol, the glycoside shows no activity, the free substance clears radical quenching properties (data not shown).

Discussion

Since oxidative stress is a common pathomechanism of inflammatory reactions and several other diseases of the gastrointestinal tract (Aiko and Grisham, 1995; Grisham, 1994; Keshavarzian et al., 1992; Kolios et al., 1998; Collins et al., 2001), it is interesting to study whether therapeutic drugs reveal antioxidative properties as e.g. shown already for rebamide or lansoprazole (Hahm et al., 1997; Iinuma et al., 1998; Suzuki et al., 1995), respectively.

Here we show that the phytopharmacon STW 5 and its constitutent plant extracts reveal strong antioxidative properties which in the presently used systems are at least comparable to that of well-known antioxidants. Besides free radicals produced by the thermolytic decomposition of AAPH, mainly superoxide anions and related radicals were the targets in the other systems. It is obvious that especially an extract from peppermint leaves has a very potent radical quenching potential; however, when other indicator reactions are used, quite different relations can be found (Schempp et al., 2004, 2006). In general, literature can be found demonstrating antioxidative activities of all extracts and many main phenolic compounds present in STW 5 (for review see Germann, 2005).

The differences in the activities found in the various complex cellular systems suggest, that the antioxidative effects are not only due to direct quenching reactions but also, at least in part, to specific inhibitory interaction with the metabolic pathways leading to the cellular secretion of free radicals. In addition, these results show, that in the cellular systems the whole phytopharmacon reveals supraadditive effects in comparison with the single extracts. Supraadditive effects can be explained by the increased possibility of interactions that allows more synergistic or cooperative effects between the various compounds involved. Similar observations were described also in other systems (Liu, 2003, 2004).

Another point of interest was the comparison between the effects of whole extracts and those of isolated main compounds present in the different extracts. All examined examples show significant differences with usually pronounced antioxidative effects of the extracts while not all of the isolated compounds show this effect. Both observations lead to the conclusion that especially the antioxidative action of plant extracts is strongly influenced from the mixture and composition of the extract and that isolated compounds have a lower over all activity.

The dose–response relations of some isolated compounds present within the different extracts show that many of the natural substances are more potent in radical quenching than the standard antioxidant trolox. The knowledge of these activities and their further characterization, also with respect to their therapeutic potential, could help to find improved strategies for the treatment of inflammation.

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