*Full Paper***Effect of an Herbal Preparation, STW 5, in an Acute Model of Reflux Oesophagitis in Rats**

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Abstract. A multitarget herbal preparation, STW 5, has been used clinically in different gastrointestinal disorders including functional dyspepsia and irritable bowel syndrome. Previous studies have shown that it possesses properties that may render it useful in gastro-oesophageal reflux disease (GERD). We performed this study to test this compound in an acute model of reflux oesophagitis in rats. Oesophagitis was induced surgically by ligating the pyloric end and fore-stomach. Lower oesophageal pH was measured 3 h later in conscious animals. Five hours after surgery, animals were sacrificed and the oesophagi were examined macroscopically and histologically. Selected markers of inflammation were measured in oesophageal homogenates. STW 5 was given orally for 5 days before induction of oesophagitis. Pantoprazole was used as a reference standard. Ligated animals showed a high incidence of ulcerative lesions associated with a marked increase in myeloperoxidase, thiobarbituric acid-reactive substances, tumor necrosis factor- α , and interleukin-1 β . STW 5 did not affect oesophageal pH, but dose-dependently reduced the severity of the oesophageal lesions and normalized the deranged level of the inflammation markers. The beneficial effects were confirmed histopathologically. STW 5 proved to be effective in protecting against inflammatory lesions in this model of oesophagitis, thus warranting further investigation of its potential therapeutic usefulness in GERD.

Keywords: pyloric ligation, rat model, reflux oesophagitis, STW 5, gastro-oesophageal reflux disease

Introduction

Gastro-oesophageal reflux disease (GERD) may be described as a condition that develops when reflux of gastric contents into the oesophagus leads to troublesome symptoms, involving mucosal damage and its sequelae. The incidence of GERD is estimated to be 10% – 20% in Western countries, making it one of the most prevalent gastrointestinal disorders (1). Although proton pump inhibitors (PPI) have markedly improved the management of GERD, 30% – 40% of GERD patients continue to suffer from symptoms during PPI treatment (2). This

fact together with recent safety concerns regarding their long-term use (3) makes it necessary to search for effective and safe alternatives.

Recent investigations indicate that the mucosal damage in GERD is due to several causative agents in the refluxate (4, 5) that stimulate mucosal and sub-mucosal cells to release mediators, eliciting an inflammatory reaction and leading to visceral hypersensitivity and to other symptoms of GERD (6, 7). Inflammatory processes seem to play a key role in the underlying mechanisms of the symptoms and pathogenesis of other gastrointestinal conditions as well, such as functional dyspepsia (FD) and irritable bowel syndrome (IBS) (8). Because of the overlap in symptoms and pathogenesis between GERD, FD, and IBS (9 – 13), it might be expected that drugs effective in one condition might also show beneficial activity

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in related ones.

A potential alternative to PPIs for the treatment of GERD is the use of herbal preparations. An herbal multi-component preparation, STW 5, containing well-defined standardized extracts of *Iberis amara* (*Brassicaceae*), *Angelica archangelica* root (*Apiaceae*), *Matricaria chamomilla* flower (*Asteraceae*), *Mentha piperita* leaves (*Labiatae*), *Carum carvi* fruits (*Apiaceae*), *Silybum marianum* fruits (*Asteraceae*), *Melissa officinalis* leaves (*Lamiaceae*), *Chelidonium majus* (*Papaveraceae*), and *Glycyrrhiza glabra* root (*Fabaceae*) (14, 15) was found to be very effective in the treatment of patients with FD as well as in IBS (16–22). Its mechanism of action seems to be multi-factorial, including anti-inflammatory and mucosal protective effects (23, 24). It was also shown to improve gastric accommodation (25, 26), reduce visceral hypersensitivity (27, 28), and increase the tone of the lower oesophageal sphincter (LES) (29), all of which are factors that play an important role in the pathogenesis of GERD (30).

These facts have led us to conduct the present study to investigate the effectiveness of STW 5 in experimental oesophagitis and to probe further into its possible mechanism of action.

Materials and Methods

Drugs

STW 5 was generously provided in the form of its commercial preparation (Iberogast®, batch number 720843) by Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany. The preparation is provided as a tincture containing 31% alcohol. Pantoprazole was purchased from Altana AG (Wesel, Germany).

Animals

Adult male Wistar rats, weighing 150–200 g each, were obtained from the Modern Veterinary Office for Laboratory Animals, Cairo, Egypt and left to acclimatize for one week at the animal facility of the Faculty of Pharmacy, Cairo University, before submitting them to experimentation. They were provided with the standard pellet diet, given water ad libitum, and kept at a temperature of $22 \pm 3^\circ\text{C}$ and a constant relative humidity throughout the experimental period. The study was carried out according to The European Communities Council Directive of 1986 (86/609/EEC) and approved by the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University.

Induction of reflux oesophagitis

Reflux oesophagitis was induced according to the method of Yamato et al. (31) with minor modifications.

Briefly, animals were deprived of food for 18 h prior to surgery. Under ether anesthesia, the abdomen was incised along the midline and both the pyloric end of the stomach and limiting ridge (transitional region between the forestomach and corpus) were ligated carefully with a 2-0 silk thread. The incised regions were rapidly sutured and the animals were allowed to recover in their cages. A separate group of animals were only sham-operated, whereby they were anesthetized, their abdomens opened, and then sutured again without ligating their stomachs.

Experimental design

The rats were blindly allocated to several groups assigned as follows:

1. STW 5-treated groups, given STW 5 in doses of 0.2, 0.5, or 2 ml/kg respectively
2. Vehicle-treated group, given the same volume of 31% ethanol equivalent to the amount found in STW 5 administered to the treated groups
3. Sham-operated control, treated with vehicle as above
4. Pantoprazole-treated group, given pantoprazole as a reference standard in a dose of 5 mg/kg

All drugs were given by oral gavage once daily for 5 successive days. On day 5, 3 h after the last drug administration, oesophagitis was induced as described above. At 3 h after surgery, at the beginning of visible reflux (foaming around the mouth), the pH in the lower third of the oesophagus was determined in conscious animals using a 1 mm antimony probe and an iPH long-term pH-meter (Standard Instruments, Karlsruhe, Germany).

After a further 2 h (5 h from surgery), the animals were sacrificed by cervical dislocation and the lower 3 cm of the oesophagus excised, opened longitudinally, and examined macroscopically for signs of ulceration and/or perforations. The area of ulceration was measured and expressed as a percentage of the total oesophageal tissue.

The oesophagus samples were either fixed in 10% formalin and used for histopathological examination or homogenized with ice-cold phosphate buffer (pH 7.4) to prepare 10% homogenates and stored at -20°C until processed further.

Determination of inflammation markers

One aliquot of homogenate was used for estimation of thiobarbituric acid-reactive substance (TBARS), as a measure of lipid peroxidation, using the method of Uchiyama and Mihara (32). Another aliquot was freeze-thawed twice and then sonicated in presence of 0.5% hexadecyltrimethylammonium bromide to release myeloperoxidase (MPO) from neutrophils; this was used for estimation of MPO activity kinetically by measuring the

rate of hydrogen peroxide-dependent oxidation of *o*-dianisidine catalyzed by MPO (33). Two other samples of the homogenate were used to assay tumor necrosis factor (TNF)- α and interleukin (IL)-1 β levels by enzyme linked immunosorbent assay (ELISA) using rat-specific kits (Quantikine; R&D Systems, Wiesbaden-Nordenstadt, Germany).

Histopathological examination

The oesophagi of 4 to 6 animals, randomly chosen from each group, except for the groups treated with the lower doses of STW 5, were fixed in 10% formalin and embedded in paraffin. Six- μ m-thick sections were stained with hematoxylin-eosin and examined under a Leica Aristoplan microscope (Leica, Bensheim, Germany). Images were captured with a charge-coupled device camera (Visitron Systems, Puchheim, Germany).

Statistics

Results are expressed as the mean $\pm$ S.E.M. Differences between groups were compared by one way analysis of variance (ANOVA) followed by Tukey Kramer's multiple comparison test. The level of significance was taken at $P < 0.05$.

Results

Reduction of macroscopic lesions by STW 5

At 5 h after ligation of the pylorus and fore-stomach, severe hemorrhagic lesions had developed in the oesophagus of the animals (Fig. 1), ca. 50% of which showing overt perforations. Pre-treatment with STW 5 significantly reduced the area and depth of these lesions in a dose-dependent manner. This was reflected by a reduction in the percentage of ulcerative area as compared to the total area of oesophageal tissue (Fig. 2) and a decrease in the number of animals showing perforations (Fig. 3). The effect of 2 ml/kg STW 5 was similar to that of 5 mg/kg pantoprazole, which was used as a reference drug.

Protective effect of STW 5 against changes in inflammation markers

Ligated animals showed a threefold increase in tissue MPO activity, a measure for neutrophilic infiltration, as well as more than a twofold elevation of TBARS, a measure of lipid peroxidation, indicating the involvement of both processes in the development of oesophagitis in this model. STW 5 was effective in normalizing the changes in both parameters in a dose-dependent manner (Figs. 4 and 5), complete normalization being achieved with the dose of 2 ml/kg. Thus, the drug showed good anti-inflammatory and good anti-oxidant properties, two

Fig. 1. Macroscopic examination of oesophagi from sham-operated and from ligated rats before and after treatment with STW 5. A: Oesophagus of a vehicle-treated ligated rat showing a large area of lesions with bleeding and necrosis. B: Oesophagus of a ligated rat treated with 0.5 ml/kg STW 5 showing some lesions. C: Oesophagus of a ligated rat treated with 2 ml/kg STW 5 showing reddening but no lesions. D: Intact oesophagus from a sham-operated rat.

Fig. 2. Reduction of the ulcerative area in rats exposed to reflux oesophagitis after pre-treatment with STW 5. Rats were treated with STW 5, vehicle, or pantoprazole (5 mg/kg) for 5 successive days. Reflux oesophagitis was induced on day 5 by ligation of the pylorus and fore-stomach 5 h before sacrifice. Data are expressed as the mean $\pm$ S.E.M. for 8–14 animals. [#] denotes significant difference from sham-operated animals, ^{*} denotes significant difference from the ligated control, $P < 0.05$.

important aspects that play a contributing role towards its therapeutic usefulness. Ligated animals also showed a more than threefold increase in $\text{TNF-}\alpha$ (Fig. 6), and an even more dramatic rise in $\text{IL-1}\beta$ concentration in the

oesophageal tissue (Fig. 7). Treatment with STW 5 was again effective in reversing all these changes in a dose-dependent manner, reaching full normalization with the

Fig. 3. Reduction in the incidence of perforations in the oesophagus of pylorus-ligated rats following pre-treatment with STW 5. Rats were treated with STW 5, vehicle, or pantoprazole (5 mg/kg) for 5 successive days. Reflux oesophagitis was induced on day 5 by ligation of the pylorus and fore-stomach 5 h before sacrifice.

Fig. 4. Dose-dependent reduction in MPO activity in the oesophageal tissue of rats with reflux oesophagitis after pre-treatment with STW 5. Rats were treated with STW 5, vehicle, or pantoprazole (5 mg/kg) for 5 successive days. Reflux oesophagitis was induced on day 5 by ligation of the pylorus and fore-stomach 5 h before sacrifice. Data are expressed as the mean $\pm$ S.E.M. for 6–8 animals. # denotes significant difference from sham-operated animals, * denotes significant difference from the ligated control, $P < 0.05$.

Fig. 5. Dose-dependent reduction in the concentration of TBARS in the oesophageal tissue of rats with reflux oesophagitis after pre-treatment with STW 5. Rats were treated with STW 5, vehicle, or pantoprazole (5 mg/kg) for 5 successive days. Reflux oesophagitis was induced on day 5 by ligation of the pylorus and fore-stomach 5 h before sacrifice. Data are expressed as the mean $\pm$ S.E.M. for 6–8 animals. # denotes significant difference from sham-operated animals, * denotes significant difference from the ligated control, $P < 0.05$.

Fig. 6. Dose-dependent reduction in the concentration of $\text{TNF-}\alpha$ in the oesophageal tissue of rats with reflux oesophagitis following pre-treatment with STW 5. Rats were treated with STW 5, vehicle, or pantoprazole (5 mg/kg) for 5 successive days. Reflux oesophagitis was induced on day 5 by ligation of pylorus and fore-stomach 5 h before sacrifice. Data are expressed as the mean $\pm$ S.E.M. for 6–8 animals. # denotes significant difference from sham operated animals, * denotes significant difference from the ligated control, $P < 0.05$.

Fig. 7. Reduction in IL-1 β concentration in the oesophageal tissue of rats with reflux oesophagitis after pre-treatment with STW 5. Rats were treated with STW 5, vehicle, or pantoprazole (5 mg/kg) for 5 successive days. Reflux oesophagitis was induced on day 5 by ligation of the pylorus and fore-stomach 5 h before sacrifice. Data are expressed as the mean $\pm$ S.E.M. for 6–8 animals. # denotes significant difference from sham-operated animals, * denotes significant difference from the ligated control, $P < 0.05$.

dose of 2 ml/kg, comparing favourably with pantoprazole.

STW 5 does not affect oesophageal pH

In contrast to pantoprazole, STW 5 did not affect the pH measured in the lower third of the oesophagus 3 h after ligation. Both doses of STW 5 showed values similar to those measured in control ligated animals (Fig. 8).

Protection against histopathological changes by STW 5

Microscopy of oesophageal samples from sham-operated rats revealed intact mucosa, sub-mucosa, and muscularis (Fig. 9a). Vehicle-treated ligated animals showed extensive epithelial defects, leukocyte infiltrates in lamina propria, and sub-mucosa as well as oedema (Fig. 9b and c). There were almost no such changes in both STW 5- and pantoprazole-treated groups (Fig. 9d and e, respectively).

Discussion

Providing basic evidence for the therapeutic efficiency of drugs depends primarily on knowledge of underlying mechanisms of disease and rationalization of treatment accordingly. GERD is one of the most common conditions met with in gastroenterology and is mainly treated with PPIs by virtue of their inhibitory effect on gastric acidity. However, suppressing gastric acid is not physi-

Fig. 8. Inhibition of pH in the lower third of the oesophagus of rats following pyloric ligation remains unaffected by prior treatment with STW 5. Rats were treated with STW 5, vehicle, or pantoprazole (5 mg/kg) for 5 successive days. Reflux oesophagitis was induced on day 5 by ligation of the pylorus and fore-stomach 5 h before sacrifice. Data are expressed as the mean $\pm$ S.E.M. for 6–8 animals. # denotes significant difference from sham-operated animals, * denotes significant difference from the ligated control, $P < 0.05$.

ological and does not affect the underlying causes of the mucosal damage. As a result, the disease usually relapses and most patients need long-term treatment (34). Such long term use of PPIs has recently been associated with a multitude of side effects, including nutritional deficiencies and an increased incidence of infection, gastric or colon malignancies, osteoporosis-related fractures, acute coronary syndromes, myopathy, as well as acute interstitial nephritis and rebound acid hypersecretion that could lead to PPI-dependency (3, 35–39). It should also be emphasized that the response to PPIs is not always satisfactory, especially in non-erosive reflux disease, and as many as 30%–40% of GERD patients continue to experience symptoms despite of the use of high doses of PPIs (2). There is therefore a constant search for alternative medication with good efficacy and fewer side effects. STW 5 is an herbal preparation used in FD and IBS. Several studies undertaken to elucidate its mechanism of action showed that it acts by affecting multiple targets, some of which being also relevant for reflux patients. This has raised our interest to carry out basic experiments to explore its potential usefulness in this condition, as well as its mechanism of action.

In the present study, STW 5 was shown to reduce the morphological signs of reflux oesophagitis as well as the associated changes in inflammation markers as efficiently as pantoprazole.

Several pathological mechanisms have been suggested to play a role in reflux oesophagitis, including hypersen-

Fig. 9. Representative samples of the examined oesophageal sections. A: Oesophagus from a sham-operated rat showing intact layers. B: Epithelial necrosis, inflammatory infiltrates, and massive oedema of mucosa and sub-mucosa in a vehicle-treated ligated rat. C: Extensive leukocyte infiltrates in the oesophageal sub-mucosa of a vehicle-treated ligated rat. D: Normal appearance of mucosa, sub-mucosa, and muscularis of the oesophagus of a STW 5-treated ligated rat. E: Normal appearance of mucosa, sub-mucosa, and muscularis of the oesophagus of a pantoprazole-treated ligated rat (hematoxylin-eosin stain).

sitivity of the oesophageal mucosa to physiological reflux, reduced oesophageal clearance, reduced mucosal defence mechanisms, LES motility disturbances, and gastric motility disturbances, including impaired accommodation and reduced gastric emptying rate (30). In addition, recent evidence suggests that the mucosal damage observed in GERD is due to release of inflammatory mediators from mucosal and sub-mucosal cells in response to bile salts and other substances present in the gastric refluxate (7). Some of these mechanisms have been shown to be affected by STW 5, but mainly in relation to other pathological conditions. In a sub-group analysis in patients with FD, it was found to relieve heartburn and other acid-related symptoms (40). It was also reported to improve gastric accommodation in guinea pigs as well as in humans (25, 26), to reduce hypersensitivity to both mechanical and chemical stimuli in rat ileum in-situ (27, 28), to enhance LES contractility (29), and to reduce acid output and improve mucosal protection (23, 24).

Because GERD is a disease with an intricate multifactorial pathophysiology, no single experimental model can completely mimic the human situation. Since in the present study, we were interested in investigating the drug's effectiveness in experimental oesophagitis with

special focus on its anti-inflammatory and mucosal protecting effects, we chose an experimental model in which gastric accommodation, visceral hypersensitivity, and LES tone do not play any significant role in the induction of oesophagitis or any observed improvement. The choice of model was intended to exclude any of these factors, which were shown in previous studies to be affected by STW 5 (25 – 29) and to focus mainly on its suggested abilities to protect from inflammation, enhance mucosal protection, and reduce acid output.

Our findings show that the drug reduced macroscopically the area and severity of the lesions and guarded against associated histopathological changes. Furthermore, it affected several parameters conducive of an anti-inflammatory effect.

In a rat model of acute reflux oesophagitis, Yamaguchi et al. (41) showed that induction of this condition led to an increase in the levels of TBARS (an index of lipid peroxidation), MPO activity (an index of neutrophilic infiltration), and TNF- α (an inflammatory mediator) among other parameters measured. These authors concluded that ROS and lipid peroxidation mainly derived from neutrophils, which are stimulated and mobilized by TNF- α , are implicated in the oesophageal inflammation induced by the reflux of the gastro-duodenal contents.

The involvement of TNF- α and IL-1 β has been substantiated by other authors as well (42). In patients with reflux oesophagitis and Barrett's oesophagus, ROS seem also to play a dominant role (43, 44). In our experiments, however, treatment with STW 5 normalized the elevated levels of TBARS, MPO, TNF- α , and IL-1 β in the oesophageal tissue, indicating both an anti-oxidant as well as an anti-inflammatory effect.

These findings are in accordance with published data, showing that STW 5 possesses potent antioxidant activity in several models of oxidative stress, by virtue of its radical-scavenging activity as well as its ability to specifically inhibit metabolic pathways leading to cellular secretion of free radicals, such as inhibition of MPO activity (45, 46). The beneficial effects of STW 5 could partly be attributed to the flavonoid content of its different constituents (47). Rao and Vijayakumar (48), in a model of oesophagitis basically similar to the one used here, reported that quercetin significantly inhibited lipid peroxidation and increased the glutathione level and suggested that antioxidants could attenuate the severity of reflux oesophagitis and prevent the oesophageal mucosal damage.

However, the effects seen in the present study cannot be explained merely by the drug's antioxidant activity, since the drug's effects on the measured inflammatory mediators TNF- α and IL-1 β were much more pronounced than its effect on TBARS as a measure of lipid peroxidation and oxidative stress.

These findings are in harmony with findings of other authors showing that STW 5 possesses direct anti-inflammatory properties *in vitro*. Bonaterra et al. (49) reported that the drug as well as some of its individual components was able to inhibit the nuclear factor-kappa B (NF- κ B) translocation into the cell nucleus of differentiated THP-1 cells after activation with lipopolysaccharide (LPS), hence inhibiting the initiation of the inflammatory cascade. This finding is of special significance, since NF- κ B activation has been associated with Barrett's inflammation and carcinogenesis in chronic GERD (50–52). The anti-inflammatory effect of STW 5 was further evidenced through inhibition of the expression and release of TNF- α in LPS-stimulated monocytes, possibly through an action on adenosine receptors (53) and inhibition of leukotriene production/release in a model of indomethacin-induced gastric ulcer (24).

In addition to the demonstrated anti-inflammatory activity, STW 5 was also shown to enhance mucin and prostaglandin E₂ secretion, thus improving mucosal protection (24).

In spite of the beneficial effects on the parameters related to reflux oesophagitis, STW 5 did not affect the oesophageal pH, in contrast to pantoprazole and to previ-

ously published data (23, 24). The dose of STW 5 used in this work was much lower than the one used as an anti-ulcerogenic agent (23) where an inhibitory effect on gastric acidity was observed with a dose of 10 ml/kg. In other experiments to study the effect of the drug on rebound acidity, it was shown that a dose of 2.5 ml/kg was able to antagonize indomethacin-induced acidity within 1 h of administration, but the effect was short-lived and wore off 3 h later (24), indicating that the effect on acidity was only transient. This would then explain the apparent lack of effect on oesophageal pH in the present work, where measurement of pH was performed 6 h after administration of the drug. The beneficial effect of STW 5 in this model could therefore be related to its anti-inflammatory effect rather than to an effect on pH.

Since GERD is a multi-factorial disease, it would probably be best treated with a multi-target drug addressing as many aspects of the disease as possible. The aim should be to restore the natural balance between aggressive and mucosal protecting factors, rather than simple acid suppression.

The present findings lend further evidence to the multifaceted therapeutic potential of STW 5 and may pave the way to a new clinical indication of the drug for the treatment of reflux disease. Furthermore, the results might help to explain the beneficial effect of STW 5 in heartburn, as a symptom of FD, a fact that could be related to an anti-inflammatory effect on the mucosa of the oesophagus.

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