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Evaluation of pharmacodynamic activities of EPs® 7630, a special extract from roots of *Pelargonium sidoides*, in animals models of cough, secretolytic activity and acute bronchitis

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ABSTRACT

Background: EPs® 7630 is a proprietary aqueous-ethanolic extract from roots of *Pelargonium sidoides* DC and has been demonstrated to dispose among others of antibacterial, antiviral, immunomodulatory, antioxidant, and tissue-protective activity. It is an approved medicinal product in more than 50 countries for the treatment of airway infections such as acute bronchitis, common cold, and sinusitis.
Purpose: While the pharmacological effects of EPs® 7630 have extensively been evaluated in diverse *in vitro* test systems, the number of publications reporting results from *in vivo* models is limited.
Study design: In the present study antitussive, secretolytic, and anti-inflammatory effects of EPs® 7630 were assessed in animal experiments following oral administration at human equivalent doses.
Methods: Antitussive effects were evaluated using ammonia- and citric acid-induced models of cough in mice (20, 40, 120 mg/kg) and guinea pigs (10, 20, 45 mg/kg), respectively. For the determination of secretolytic activity tracheobronchial secretion of intraperitoneally injected phenol red was determined in mice, while antiinflammatory action was assessed in an acute bacterial bronchitis model in rats.
Results: A significant and dose-dependent reduction of cough frequency was observed in both cough models, which was accompanied by a prolongation of cough latency time. Similarly, the extract exerted a marked secretolytic activity in mice. Induction of acute bacterial bronchitis caused characteristic histopathological changes in lung tissue adjacent to trachea and bronchi. The degree of these lesions was significantly reduced in rats treated with EPs® 7630 at doses of 30 and 60 mg/kg. This protective effect at least partially seems to be mediated by an up-regulation of superoxide dismutase and a subsequent protective effect against oxidative stress as indicated by a reduced serum level of malondialdehyde.
Conclusion: The present data further support the therapeutic use of EPs® 7630 in respiratory tract infections and provide a basis for detailed studies on its bioactive constituents as well as their *in vivo* mode of action.

Introduction

In southern Africa, different indigenous species of the plant genus *Pelargonium* are used in folk medicine for the treatment of various complaints, such as diarrhea, dysentery, liver disorders and dysmenorrhea. For more than a century, preparations from P. reniforme Curtis and/or *P. sidoides* DC. have also been used in European herbal medicine particularly for the therapy of tuberculosis and airway infections. A proprietary extract from the roots of *P. sidoides* (EPs® 7630) has been developed and registered with a full marketing authorization for the indication "acute bronchitis" by the German Federal Institute for Drugs and Medical Devices in 2005 (Brendler 2008). Therapeutic efficacy in this and related indications such as common cold, rhinosinusitis, tonsillopharyngitis, and chronic obstructive pulmonary disease has been evaluated in numerous randomized, double-blind and placebo-controlled trials as well as observational clinical study in patients from an age of one year upwards (Moyo and Van Staden 2014; Matthys et al. 2014). Safety and tolerability of EPs® 7630 has been demonstrated in 29 clinical trials and post-marketing surveillance studies involving more than 10,000 adults and children (Matthys et al. 2013).

EPs® 7630[1] is a special aqueous ethanolic extract (11% m/m; drug extract ratio 1:8–10) characterized by a high content of

Abbreviations: SOD, superoxide dismutase; MDA, malondialdehyde; SD, standard deviation.

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[1] EPs® 7630 is the active ingredient of the product Umckaloabo® (ISO Arzneimittel, Ettlingen, Germany).

epigallo- and gallocatechin based polymeric polyphenols and a broad range of extensively oxygenated 1-benzopyran-2-one derivatives (Schötz et al. 2008). *In vitro* evaluation of pharmacological activities revealed among others mild to moderate direct antibacterial and antiviral properties, inhibition of bacterial adhesion on host cells as well as activation of the innate immune defense. This later action is mediated by stimulation of release of tumor necrosis factor-alpha, nitric oxide, and interferon-beta, which are accompanied by an enhanced natural killer cell activity and an improved phagocytosis, oxidative burst and intracellular killing by human peripheral blood phagocytes. Besides, cytoprotective capabilities and an increase of ciliary beat frequency of nasal respiratory cells have been reported (Kolodziej 2011).

While the pharmacological effects of EPs® 7630 have extensively been investigated in diverse *in vitro* test systems, the number of publications reporting results from *in vivo* models is limited. In animal studies an inhibition of lipopolysaccharide-induced sickness behavior has been observed (Nöldner and Koch 2004; Nöldner and Schötz 2007), while no or only mild effects were seen on central nervous system activity in normal mice (Reynolds et al. 2004). In addition, antiviral action of EPs® 7630 has recently been confirmed in mice after inhalation of the extract (Theisen and Muller 2012). Thus, based on the main clinical indications for EPs® 7630 the present study concentrated on the evaluation of *in vivo* effects in an infective bronchitis model and two of the most common symptoms related to airway infections i.e. cough and disturbances in mucociliary clearance.

Materials and methods

Test substance

All investigations were performed with a single batch (No. 501790080/Ch.007) of the *Pelargonium sidoides* dry root extract EPs® 7630 kindly provided by the manufacturer Dr. Willmar Schwabe Pharmaceuticals, Karlsruhe, Germany. In accordance with the EPs® 7630 standard dose of 60 mg/day for adolescents and adults (body weight 15–60 kg), equivalent doses on the basis of allometric means in mice, rats and guinea pigs would be about 15–36, 7–19 and 6–15 mg/kg/day, respectively. Based on this calculation, for the animal experiments doses which approximately correspond to once, twice or three times the average human equivalent dose for an adolescent person were chosen. Hence, the following doses were applied to mice (20, 40, 120 mg/kg/day); rats (15, 30, 60 mg/kg/day) and guinea pigs (10, 20, 45 mg/kg/day). All treatments were performed by gavage. EPs® 7630 was suspended in distilled water and applied in a volume of 0.2 ml/10 g in mice and 1 ml/100 g in rats and guinea pigs, respectively.

Reference drug

For reference purpose a radix glycyrrhizae oral solution (batch No. 130503) produced by DeRun Pharmaceutical Co., Ltd., Beijing, was employed. This product is approved in China as an expectorant for upper respiratory tract infections, bronchitis, colds, and cough. Radix glycyrrhizae oral solution contains licorice liquid extract, camphor tincture and guaiac glycerol ether and has previously been shown to be active in the experimental models used in the present study. In compliance with the dose in humans (30 ml/60 kg/day), equivalent oral doses on the basis of allometric means in mice (5 ml/kg/day), rats (2.75 ml/kg/day) and guinea pigs (2.15 ml/kg/day) were selected.

Experimental animals

Male and female ICR miceSPF-class; body weight of 20 ± 2 g) as well as male and female Wistar rats SPF-class, body weight 150 ± 10 g) were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. Guinea pigs (male and female, body weight 240 ± 20 g) were purchased from Beijing Jinmuyang Laboratory Animal Breeding Co., Ltd.

Animals were kept under standardized environmental conditions (12/12 h light-dark cycle, temperature 20–26 °C, 40–70% humidity) and had access to standard feed and tap water ad libitum. They were allowed at least 24 h for adaptation. At the end of the study the animals were euthanized by cervical dislocation. All animal experiments were approved by the Medical Ethics Committee, China Academy of Chinese Medical Sciences.

Bacteria

Streptococcus pneumoniae (31001) were acquired from Chinese Pharmaceutical and Biological Products and were stored at −80 °C until use.

Chemicals

The following chemicals were purchased from the suppliers given in brackets: citric acid (Beijing Chemical Reagent Company, batch No.: 060403), ammonia (Sinopharm Chemical Reagent Co., Ltd., batch No.: 20120918), phenol red (Solarbio Company, batch No.: 20114.12.07), nutrient broth (China Pharmaceutical and Biological Products), ether (Beijing Reagent Company, batch No.: 20090707), superoxide dismutase (SOD) ELISA kit (Nanjing Jiancheng Institute of Biotechnology, batch No.: 201209), malondialdehyde (MDA) ELISA kit (Nanjing Jiancheng Institute of Biotechnology, batch No.: 201209), and micro BCA protein assay kit (Kangwei Century Company, batch No.: 201209).

Evaluation of antitussive effects

Ammonia-induced coughing in mice

A total of 59 mice were randomly distributed according to their body weight to a control group, a radix glycyrrhizae group (5.5 ml/kg), and three EPs® 7630 groups with different doses (20, 40 and 120 mg/kg). Each treatment group consisted of 12 mice with an equal number of male and female animals. The drugs were applied once daily for three consecutive days by gavage at a volume of 0.2 ml/10 g. The control group was treated under the same conditions with 0.2 ml/10 g of distilled water. One hour after the last treatment mice were placed in a container with a volume of 1 l and exposed to a vapor from 1 ml of concentrated ammonia. After 20 s the animals were removed quickly and the latency to the first cough and the total number of coughs were registered over a period of 3 min. The relative cough latency time (in %) was calculated using the following formula:

$$\text{relative cough latency time} = \frac{\text{cough latency time } (sec) \text{ of test group}}{\text{cough latency time of control group}} \times 100\%$$

Citric acid-induced coughing in guinea pigs

Suitable animals for the test were selected one day before the experiment. Guinea pigs which did not cough at all within 2 min after begin of exposure to a spray of citric acid solution or coughed less than 10 times during a 5 min observation period were discarded.

According to the body weigh a total of 50 selected guinea pigs were randomly distributed into a control group, a radix glycyrrhizae group (2.15 ml/kg), and three EPs® 7630 groups (10, 20, and 45 mg/kg). Each group consisted of 10 guinea pigs with an equal number of male and female animals. The drugs were administered intragastrically once a day at a volume of 1 ml/100 g for three consecutive days. The control group was treated under the same conditions with 1 ml/100 g of distilled water. One hour after the last dose, the guinea pigs were placed in a container with a volume of 7 l and exposed to a spray of 17.5% citric acid in water at a rate of 2 ml/min produced by a nebulizer at a constant pressure. One minute later, the latency time from begin of spraying to the first cough was registered. Thereafter,

the nebulization of citric acid solution was continued for a further 6 min and the number of coughs was subsequently registered for a period of 7 min. The relative cough latency time (%) was calculated as described above for ammonia-induced cough in mice.

Bronchosecretolytic activity

Phenol red excretion in mice

A total of 55 mice were randomly distributed to a control group, a radix Glycyrrhizae group (5.5 ml/kg), and three EPs® 7630 groups (20, 40, 120 mg/kg). Each group consisted of 11 mice with a similar proportion of male and female animals. The drugs were applied by gavage once a day at a volume of 0.2 ml/10 g for three consecutive days. Mice in the control group were treated under the same conditions with 0.2 ml/10 g of distilled water. Thirty minutes after the last dose, each mouse received an intraperitoneal injection of 0.5 ml of 2.5% phenol red solution. Half an hour later the animals were killed, the skin in the middle of the throat was cut, the tissue surrounding the trachea was removed, and a piece of trachea between the thyroid cartilage and the tracheal bifurcation was withdrawn. The trachea was rinsed five times with 2 ml 5% $NaHCO_3$ solution, and the lavage was placed into a test tube. Phenol red concentration was detected at 546 nm using a spectrophotometer.

Acute bacterial bronchitis in rats

A total of 69 rats was randomly distributed according to their body weight into a normal control group, a bronchitis control group, a radix glycyrrhizae group (2.75 ml/kg), and three EPs® 7630 groups (15, 30, 60 mg/kg). With the exception of rats in the normal control group all other animals were placed in an airtight glass container and fumigated once daily for an hour over a period of 21 days with unburned smoke from lighted cigarettes at a density of 100 g/m^3. In addition, the rats were treated intranasally once a week for a total of three times with 0.1 ml Streptococcus pneumoniae. Beginning at day 11, drugs were orally administrated daily for 10 days at a volume of 1 ml/100 g. The animals in the normal and model control groups were treated under the same conditions with 1 ml/100 g of distilled water. After overnight fasting, the rats were anesthetized with sodium pentobarbitone (60 mg/kg, intraperitoneally) on day 22. Blood was withdrawn from the abdominal aorta and placed at room temperature for 1 h. Serum was collected after centrifugation at 4 °C for 15 min at 3500 rpm.Then bronchoalveolar lavage fluid was collected. The protein content of the bronchoalveolar lavage fluid was determined and a differential cell count was obtained. The lung was removed and subjected to histopathological examination. The following criteria were applied for grading the pathological changes:

"-": Tracheal and bronchial epithelium shows no hyperplasia, no inflammation, lung tissue is normal
"+": Tracheal and bronchial epithelium have mild segmental hyperplasia, inflammation is not obvious, no pulmonary interstitial inflammation, normal structure
"++": Tracheal and bronchial epithelium has hyperplasia, surrounded by inflammation and vascular congestion
"+++": Significant increase of lymphocytes in interstitial lung tissue. Tracheal and bronchial epithelium have significant hyperplasia, surrounded by diffuse inflammation and vascular congestion.

Statistical analysis

All data, except results of histopathological examination, are expressed as the mean ± standard deviation (SD). ANOVA was used for the determination of statistical significance ($p < 0.05$). For the pairwise comparison of groups the LSD test (homogeneity of variance) or the Dunett T3 test (heterogeneity of variance) was applied. Statistical analyses were performed using SPSS software. Rank sum test was used for the statistical analysis of histopathological examination.

Results

Antitussive effects of EPs® 7630 in an ammonia-induced cough model in mice

Inhalation of ammonia vapor caused a first cough response in control animals within 25.2 ± 10.2 s and resulted in a total of 34.6 ± 19.9 coughs. Compared with the control group, cough frequency was significantly and dose-dependently reduced by up to 86% in animals receiving 20, 40, 120 mg/kg/day of EPs® 7630. At the same time cough latency time was prolonged by the treatment between 182 and 367% (Table 1).

Antitussive effects of EPs® 7630 in a citric acid-induced cough model in guinea pigs

Antitussive effects of EPs® 7630 were confirmed in a widely used second pharmacological model employing citric acid-induced cough in guinea pigs. In comparison to the control group the number of coughs was reduced by 67, 63 and 73%, respectively, in animals treated with 10, 20, 45 mg/kg/day EPs® 7630. Although the mean cough latency time was markedly prolonged in EPs® 7630 treated guinea pigs this did not reach statistical significance due to a highly variable response of individual animals in the different groups (Table 2).

Bronchosecretolytic effects of EPs® 7630 in mice

Secretolytic activity was determined in mice by the secretion of intraperitoneally injected phenol red into the tracheobronchial tract over a period of 30 min. Elimination of the dye via the respiratory tract was dose-dependently increased after administration of EPs® 7630 for three consecutive days and this effect was statistically significant at doses of 40 and 120 mg/kg/day (Table 3).

Table 1
Antitussive effects of EPs® 7630 in an ammonia-induced cough model in mice.

Group	Dose	Number of animals	Cough latency time (s)	Relative cough latency time (%)	Number of coughs
Control	–	11	25.2 ± 10.2	–	34.6 ± 19.9
Radix glycyrrhizae	5.5 ml/kg	12	39.8 ± 19.8	159	12.0 ± 8.3*
EPs® 7630	20 mg/kg	12	45.5 ± 24.5	182	9.8 ± 7.3*
EPs® 7630	40 mg/kg	12	89.0 ± 53.4*	356	5.5 ± 5.7**
EPs® 7630	120 mg/kg	12	91.8 ± 49.2**	367	4.9 ± 4.1**

Values are mean ± SD.
* $p < 0.05$ compared to control.
** $p < 0.01$ compared to control.

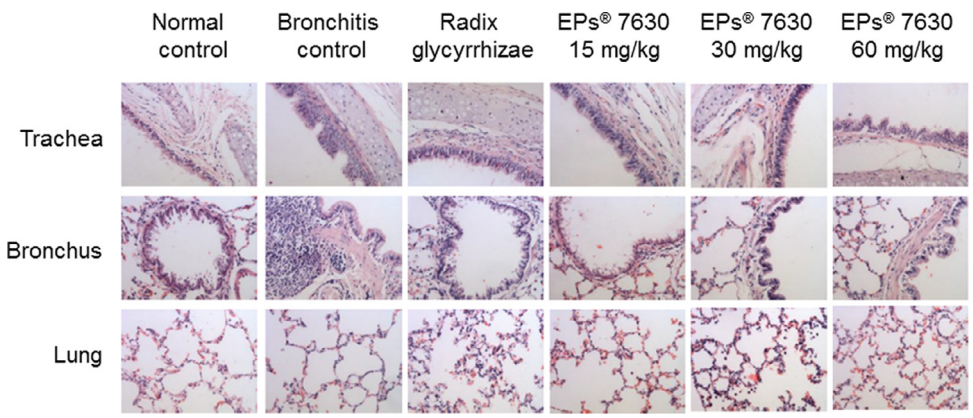

Fig. 1. Effects of EPs® 7630 on acute bronchitis in rats. Compared with the normal control group the bronchitis model control group display substantial pathological changes. Treatment with EPs® 7630 at doses of 30 or 60 mg/kg significantly reduced the tracheal lesions (see Table 4).

Table 2
Antitussive effects of EPs® 7630 in a citric acid-induced cough model in guinea pig.

Group	Dose	Number of animals	Cough latency time (s)	Relative cough latency time (%)	Number of coughs
Control	–	10	53.4 ± 20.6	–	20.0 ± 7.0
Radix glycyrrhizae	2.15 ml/kg	10	123.5 ± 65.0	231	6.7 ± 6.5**
EPs® 7630	10 mg/kg	10	111.1 ± 114.0	208	6.1 ± 5.4**
EPs® 7630	20 mg/kg	10	100.3 ± 116.9	188	7.5 ± 7.0**
EPs® 7630	45 mg/kg	10	126.2 ± 162.2	236	5.5 ± 4.3**

Values are mean ± SD.
** $p < 0.01$ compared to control.

Table 3
Bronchosecretolytic effects of EPs® 7630 in mice (phenol red secretion).

Group	Dose	Number of animals	Concentration of phenol red (μg/ml)
Control	–	11	274.3 ± 89.1
Radix glycyrrhizae	5.5 ml/kg	11	401.6 ± 104.4**
EPs® 7630	20 mg/kg	11	349.1 ± 59.9
EPs® 7630	40 mg/kg	11	414.1 ± 113.1**
EPs® 7630	120 mg/kg	11	474.5 ± 116.7**

Values are mean ± SD.
** $p < 0.01$ compared to control.

Table 4
Effects of EPs® 7630 on pathological changes in tracheae of rats with acute bacterial bronchitis.

Group	Dose	Number of animals	Degree of tracheal lesions				
			–	+	++	+++	*p*-value
Normal control	–	12	12	–	–	–	
Bronchitis control	–	11	–	–	5	6	
Radix glycyrrhizae	2.75 ml/kg	11	2	5	2	2	<0.05
EPs® 7630	15 mg/kg	12	–	6	4	2	>0.05
EPs® 7630	30 mg/kg	11	–	6	4	1	<0.01
EPs® 7630	60 mg/kg	12	–	6	6	–	<0.05

Table 5
Effects of EPs® 7630 on pathological changes in bronchi of rats with acute bacterial bronchitis.

Group	Dose	Number of animals	Degree of bronchial lesions				
			–	+	++	+++	*p*-value
Normal control	–	12	12	–	–	–	
Bronchitis control	–	11	–	4	5	2	
Radix glycyrrhizae	2.75 ml/kg	11	–	7	3	1	>0.05
EPs® 7630	15 mg/kg	12	–	9	2	1	>0.05
EPs® 7630	30 mg/kg	11	–	8	2	1	>0.05
EPs® 7630	60 mg/kg	12	–	9	2	1	>0.05

Effect of EPs® 7630 on acute bacterial bronchitis in rats

Daily inhalation of unburned smoke over a period of 21 days in combination with weekly intranasal applications of Streptococcus pneumoniae had no effect on the number of leucocytes and the protein content in bronchoalveolar lavage on day 22 when compared to normal control rats. Likewise, these parameters were not affected by daily oral administration of EPs® 7630 (15, 30 and 60 mg/kg/day) from day 11 to day 21 (data not shown). However, histopathological examination revealed significant pathological changes around the tracheae and bronchi in lungs of model group animals (Fig. 1). The degree of lesions in the tracheae was significantly reduced in rats treated with EPs® 7630 at the intermediated and high dose (Table 4), while no significant effect on bronchial histopathology was seen (Table 5).

Induction of bronchitis was accompanied by a strong increase of SOD activity in the serum. Obviously, up regulation of this defense mechanism was sufficient to counteract a possible inflammation-induced oxidative damage of lipids as determined by the MDA concentration in the serum. Interestingly, treatment of rats with EPs® 7630 led to a further rise of SOD and a reduction of MDA levels even below those in healthy control animals (Table 6).

Discussion

Acute airway infections are among the most common reasons for consultation of a physician as well as absence from school or work due to illness. In the majority of cases respiratory infections are caused by viruses (e.g. adenoviruses, rhinoviruses, influenza and parainfluenza viruses, coronaviruses virus, respiratory syncytial virus, coxsackie viruses). They are usually self-limiting and in two thirds of cases will be cured within two weeks (Holzinger et al. 2014). Thus,

Table 6
Effects of EPs® 7630 on concentration of SOD and MDA in serum of rats with acute bacterial bronchitis.

Group	Dose	Number of animals	SOD (U/ml)	MDA (nmol/ml)
Normal control	–	12	99.5 ± 62.1	4.66 ± 1.47
Bronchitis control	–	11	188.2 ± 82.1##	4.49 ± 1.09
Radix glycyrrhizae	2.75 ml/kg	11	128.8 ± 110.9	4.44 ± 0.96
EPs® 7630	15 mg/kg	12	242.3 ± 40.1	4.36 ± 1.16
EPs® 7630	30 mg/kg	11	263.1 ± 62.1*	4.49 ± 1.03
EPs® 7630	60 mg/kg	12	213.0 ± 79.2	3.53 ± 0.83*

Values are mean ± SD.
* $p < 0.05$ compared to bronchitis control.
$p < 0.01$ compared to normal control.

relief of symptoms and shortening of disease duration is the primary aim of medicinal treatment.

Viral infections of the respiratory tract ("common cold") are usually accompanied by an acute cough. Other symptoms include sore throat, runny nose, headache, growing pains, fatigue, and possibly fever. The transition from a common cold to an acute bronchitis is fluent. In the early stages of the inflammatory reaction patients are particularly afflicted by a dry cough. Following the release of inflammatory mediators or damage of the airway epithelium sensory nerves are activated which initializes the cough reflex. Although cough is normally a defensive reflex that keeps the respiratory tract free of obstructions and harmful substances it is non-productive at this stage as mucus secretion is not yet increased. Due to its pure force coughing bouts further irritate the already affected mucous membranes and induce a vicious circle. Thus, antitussive therapy is indicated at this phase of acute airway infections (Holzinger et al. 2014; Reynolds et al. 2004).

Although the beneficial therapeutic effects of EPs® 7630 in the treatment of respiratory tract infections has been demonstrated in a great number of randomized controlled clinical trials and are well supported by its until now described pharmacological profile, possible antitussive activity has not yet been investigated. In order to simulate the situation in the early stages of an acute bronchitis, a dry, non-productive cough was induced by chemical stimulation of sensory nerve receptors following inhalation of ammonia and citric acid in mice and guinea pigs, respectively. The results show that EPs® 7630 at therapeutically relevant oral doses significantly cuts down cough frequency and also extends relative cough latency time in a dose-dependent manner in both species investigated.

Depending on their mode of action, medications used for the treatment of cough are usually classified as antitussive or protussive. Antitussive agents suppress the cough reflex peripherally by suppressing the stimulation of sensory neurons in the airways or centrally at the level of the cough center in the nucleus tractus solitarius and its integrating network (Reynolds et al. 2004). Protussive compounds improve the effectiveness of cough by improving the expectoration of airway secretion by increasing the volume of secretion (secretolytics), lowering the viscosity of mucus (mucolytics) or stimulation of mucociliary clearance by enhancing ciliary activity and mucin transport (secretomotorics).

With respect to citric acids-induced cough considerable similarity in conscious guinea pigs and men has been reported and a broad range of antitussive as well as protussive drugs have been observed to be active in this model, e.g. opioids, local anesthetics, histamine H1 receptor antagonists, 5-HT3 receptor agonists, β2-adrenoceptor agonists, antimuscarinics expectorants and mucolytics. Currently it is not known by which mechanism(s) EPs® 7630 exerts its cough inhibiting action. However, a central mode of action may not be excluded as EPs® 7630 previously has been demonstrated to suppress sickness behavior, a coordinated adaptation of behavior that develops during an infection in response to the central nervous action of inflammatory cytokines (Nöldner and Schötz 2007).

While cough in the early stages of an airway infection is considered as non-productive it is classified as productive with an ensuing increase of mucus production and expectoration. Stimulation of airway secretion is a first line-defense mechanism to clear the respiratory tract of foreign matter. Thus, it was considered worthwhile to evaluate if EPs® 7630 may also have an effect on tracheobronchial secretion. Measurement of secretolytic activity was evaluated by measuring the excretion of phenol red into respiratory tract (Coppi and Gatti 1989). Indeed, the extract was found to dose-dependently increased elimination of the dye via the respiratory tract with statistically significant effects at doses of 40 and 120 mg/kg. Thus, secretolytic activity could also contribute to the cough inhibiting action of EPs® 7630 by dilution and accelerated removal of the chemical stimuli whereby the later effect may be enhanced by the previously reported increase of ciliary beat frequency in the presence of the extract (Neugebauer et al. 2005).

Previously, concern has been expressed that antitussive therapy should not be combined with the application of protussive drugs due to the possibility that excessive respiratory secretion may accumulate in the airways. However, modern treatment concepts envisage a combination of antitussive and secretomucolytic activity since respiratory tract infections are rarely associated with a substantial mucus production and ongoing cough, particularly during the night, may be extremely debilitating for affected patients (Morice et al. 2002).

Besides its beneficial effects in acute bronchitis (Matthys and Kamin 2011), EPs® 7630 has previously been shown to prolong time to exacerbation of chronic bronchitis and quality of life in patients with chronic obstructive pulmonary disease (Matthys et al. 2010).Thus, the present preclinical study on *in vivo* activity of EPs® 7630 after oral application was supplemented by examining its effect in a bacterial bronchitis in rat as well. Although daily inhalation of unburned smoke over a period of 21 days in combination with weekly intranasal applications of Streptococcus pneumoniae had no effect on the number of leucocytes and the protein content in the bronchoalveolar lavage, histopathological examination clearly demonstrated an ongoing inflammatory reaction. These pathological changes were dose-dependently reduced around the tracheae of treated animals when compared with the model control group while a significant effect on bronchial histopathology was only seen at a dose of 30 mg/kg. This protective effect at least partially seems to be mediated by an up regulation of SOD and a subsequent protective effect against oxidative stress as indicated by a reduced serum level of MDA.

A wide selection of compounds has been isolated and identified in EPs® 7630 (Schötz and Nöldner 2007). Characteristic constituents of the extract are highly oxygenated coumarins, simple phenols (e.g., catechin, gallocatechin) and high molecular weight proanthocyanidins. In general, the coumarins and polymeric polyphenols are described as the main pharmacologically active agents in EPs® 7630

(Kolodziej 2007, 2011). However, due to their high degree of polymerization it has been questioned if the proanthocyanidins may at all contribute to the pharmacological action of EPs® 7630 after oral administration (Kolodziej 2007). Thus, the present experiments were designed to investigate antitussive, secretolytic and anti-inflammatory effects of EPs® 7630 in animal experiments following oral administration of human equivalent doses. The now generated data definitely support the oral therapeutic use of EPs® 7630 in respiratory tract infections and provide a basis for further studies on its bioactive constituents as well as their *in vivo* mode of action.

Conflict of interest

Yanyan Bao, Yingjie Gao, Xin Pan, Yahong Jin and Xiaolan Cui are employees of Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China, and do not have any conflict of interest. Egon Koch is employee of Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe (Germany).

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