Antioxidant Profiles of a Prepared Extract of Chinese Herbs for the Treatment of Atopic Eczema

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A standardized mixture of Chinese herbs, Zemaphyte®, taken orally as a daily decoction has been shown to be effective in the treatment of atopic eczema. This study showed that Zemaphyte® is an efficient antioxidant, being capable of scavenging both superoxide and hydroxyl, and preventing peroxidation of biological membranes. It does not degrade hydrogen peroxide directly, but instead most likely forms a Zemaphyte–hydrogen peroxide complex. The complexed hydrogen peroxide can then be degraded in the presence of catalase to form oxygen and water. It is conceivable that Zemaphyte® may contribute to the down-regulation of the activities of cells implicated in atopic eczema through its antioxidant activities. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: antioxidant; atopic eczema; dermatitis; Chinese herbal medicine; reactive oxygen species; Zemaphyte®.

INTRODUCTION

Atopic eczema, a common inflammatory condition of the skin, often appears first in early infancy and may persist into adulthood. Although it is not life threatening, it affects the quality of life (Fivenson et al., 2002). The clinical features include itching, redness and scaling of the skin. Histologically, it is typified by the presence of a predominantly lymphohistocytic infiltrate around the upper dermal vessels, together with spongiosis. It is usually associated with increased levels of circulating immunoglobulin E (IgE) in the blood and mast cells in the lesions but they appear to be incidental to the condition.

Standard treatments for atopic eczema include the regular use of emollients and topical corticosteroids. For severe cases ultraviolet B (UVB), psoralen photochemotherapy (PUVA) (Atherton et al., 1988), systemic corticosteroids (Heddle et al., 1984) and cyclosporine (Sowden et al., 1991) are often prescribed. Recently macroside immunosuppressants, tacrolimus (FK-506) and piperolimus (SDZ-ASM 981), with a cyclosporine-like mechanism of action have been introduced for the treatment of moderate to severe atopic eczema (Ashcroft et al., 2005).

Traditional Chinese medicine (TCM) as an alternative form of treatment for atopic eczema has been highly significant because of considerable interest by patients (Artik and Ruzicka, 2003; Koo and Desai, 2003). One of the remedies, a daily decoction of a mixture of Chinese herbs taken orally, is of particular importance (Atherton et al., 1991). This was standardized to contain 10 Chinese herbs and is known as Zemaphyte® (Phytopharm Plc., Cambridge, UK). Zemaphyte® gave impressive responses in both children and adults (Sheehan and Atherton, 1992; Sheehan et al., 1992), however, it had also been reported that it did not appear to benefit Chinese patients with recalcitrant atopic eczema in a controlled trial (Fung et al., 1999). Systematic reviews on these clinical trials suggest Zemaphyte® may be effective, but concluded that the randomized controlled trials were small and poorly reported, and the results were heterogeneous (Armstrong and Ernst, 1999; Zhang et al., 2006). Further well-designed and larger scale trials were recommended.

It had been postulated that the clinical efficacy of Zemaphyte® was due to its ability to intercept and down-regulate many immunological pathways implicated in the pathogenesis of atopic eczema. It causes the decrease in Langerhans cells, circulating serum IgE complexes and vascular adhesion molecules (Xu et al., 1997). Moreover, it inhibits the expression of CD23, a low affinity IgE receptor on peripheral blood monocytes, implicated in the pathogenesis of atopic eczema (Banerjee et al., 1998).

Recent evidence suggests that atopic eczema patients may be under oxidative stress as a result of the inflammatory responses induced by elevated levels of inflammatory cytokines, adhesion molecules and chemotactic factors, apart from immune pathway disturbance (Briganti and Picardo, 2003). These patients may have polymorphonuclear monocytes and granulocytes that are hyper-reactive, which produce reactive oxygen species more easily than control subjects. Hence it is believed antioxidants may be beneficial in conditions of oxidative stress and have been shown to reduce PMN oxidative burst and other functions (Briganti and Picardo, 2003; Kirby and Schmidt, 1997). Kirby and Schmidt postulated that the activity of Zemaphyte® may be attributed to its antioxidant properties on the skin. Using a diphenylpicrylhydrazyl (DPPH) assay and superoxide scavenging activity, Zemaphyte® possessed higher antioxidant activity compared with the placebo mixture (Kirby and Schmidt, 1997).
Zemaphyte® is now classified as a ‘drugs and other substances not to be prescribed to patients’ by the UK National Health Services and is no longer being manufactured (Zhang et al., 2006). However, the herbs present in the formulation are widely available in TCM and continue to be used as generic mixtures for the treatment of atopic eczema (Artik and Ruzicka, 2003; Koo and Desai, 2003). Formulations (P07P and PYM00217 extracts) using three of the original ten plants were evaluated for canine atopic dermatitis and are now marketed as Phytopica® by Phytopharm plc. (Ferguson et al., 2006). In this study, the antioxidant properties of Zemaphyte® were further evaluated for its scavenging activities on superoxide, hydroxyl radical and hydrogen peroxide, and for its ability to prevent microsomal lipid peroxidation. The implication of the observed antioxidant activities will be discussed.

MATERIALS AND METHODS

Zemaphyte® (Phytopharm Plc., Cambridge, UK) (PSE 201; 3 parts of freeze dried extract: 1 part of colloidal silica excipient in weight), a freeze dried aqueous extract, was prepared from a standardized formulation of dried plant materials commonly used in Chinese traditional medicine. The plant materials used were identified as Ledebouriella seselioides Wolff (Umbelliferae), Potentilla chinensis Ser. (Rosaceae), Clematis armandii Franch. (Ranunculaceae), Rehmannia glutinosa Libosch. (Scrophulariaceae), Paonia lactiflora Pall. (Paeoniaceae), Lophatherum gracile Brongn. (Gramineae), Dictamus dasycarpus Türez. (Rutaceae), Tribulus terrestris L. (Zygophyllaceae) and Glycyrrhiza glabra L., Schizonepeta tenuifolia Briq. (Labiatae) (Koo and Arain, 1998; Sheehan et al., 1992).

Zemaphyte® (PSE 201; 1.0 g) was mixed with redistilled water (7.5 mL), warmed at 60 °C for 10 min and centrifuged at 1000 × g for 10 min to separate the dissolved extract from the insoluble silica excipient. The supernatant was used for the antioxidant assays, and contained 100 mg/mL (10% (wt/vol)) of actual herb extract. Mannitol, ascorbic acid, uric acid, catalase (from bovine liver, ammonium sulfate and thymol free), superoxide dismutase (from horseradish, ammonium sulfate free), xanthine oxidase (grade I, from milk), horseradish peroxidase (HRP) (type I), uric acid and ascorbic acid) were freshly prepared and dissolved extract from the insoluble silica excipient. The reaction mixtures were incubated at 25 °C for 10 min to separate the dissolved extract from the insoluble silica excipient. The supernatant was used for the antioxidant assays, and contained 100 mg/mL (10% (wt/vol)) of actual herb extract.

Hydroxyl scavenging studies. A spectrophotometric procedure based on superoxide generation by xanthine/xanthine oxidase described previously was adopted (Chung, 2006). Solutions of the test samples (Zemaphyte®, uric acid and ascorbic acid) were freshly prepared and the pH adjusted to 7.4 where necessary. The inhibitory effect of Zemaphyte® on the superoxide generation system was also assessed.

Hydrogen peroxide scavenging studies. Scopoletin-HRP assay. A solution containing Zemaphyte® (0.01–1.00% (wt/vol), final concentration) and hydrogen peroxide (10⁻⁴ M, final concentration) in either redistilled water or 50 mM KH₂PO₄/KOH (pH 7.4) was incubated at a Clark’s oxygen electrode (connected to a digital model 10 controller, Rank Brothers Ltd, Botisham, Cambridge, UK) maintained at 20 °C. After pre-equilibration of 1 to 2 min, catalase (200 units/mL, final concentration) was gently introduced into the electrode cell containing the mixture to assess the level of hydrogen peroxide present. The hydrogen peroxide content was directly related to the level of oxygen released after the addition of catalase. The control consisted of hydrogen peroxide (10⁻⁴ M, final concentration) in either redistilled water or 50 mM KH₂PO₄/KOH (pH 7.4) (3 mL, final volume). The electrode was calibrated to 100% with aerated water to give optimum sensitivity.

Hydroxyl scavenging studies. The procedure of the deoxyribose assay described previously was adopted to assess hydroxyl scavenging activity of Zemaphyte® (Chung, 2006; Halliwell et al., 1987). Mannitol and ethanol were also tested as positive controls. Briefly, reaction mixtures, in a final volume of 1.0 mL, consisted of the following reagents: 20 mM KH₂PO₄/KOH (pH 7.4), 2.8 mM deoxyribose, 100 μM iron (III) chloride (FeCl₃) or 10 μM iron (III) chloride (FeCl₃)/100 μM EDTA, 1 mM hydrogen peroxide, 100 μM ascorbic acid and test compounds (Zemaphyte®, mannitol and ethanol). Solutions of FeCl₃ and ascorbic acid were freshly prepared with deaerated water before use. The reaction mixtures were incubated at 25 °C for 1 h. The pink chromogen from the oxidatively damaged deoxyribose was developed by adding 1 mL of 1% (wt/vol) thiobarbituric acid (TBA) in 0.05 M NaOH and 1 mL of 2.8% (wt/vol) trichloroacetic acid (TCA), and the mixture was heated at 100 °C for 30 min. The absorbance of the pink adduct of malondialdehyde (MDA) and TBA (MDA-TBA) was determined spectrophotometrically at 532 nm. The absorbance data were plotted as a deoxyribose competitive plot, 1/Absorbance vs concentration of test compound to allow assessment of hydroxyl scavenging activity by Zemaphyte® and the controls.

Microsomal lipid peroxidation studies. The method described previously (Chung, 2006) was adopted to determine microsomal lipid peroxidation in the presence of Zemaphyte®. Butylated hydroxytoluene (BHT) was included as the positive control. Briefly, liver microsomes (1.5 mg of protein/mL) prepared from male Wistar rats were preincubated with test compound (0.005–1.0% w/v) at 37 °C for 5 min. This was followed by addition of 0.1 mM ascorbic acid and 5 μM ferrous sulfate to initiate lipid peroxidation, and the reaction mixtures (1 mL, final volume) were incubated at 37 °C
for 3 h. The reaction was terminated by the addition of 1 mM EDTA. To assay the MDA formed during lipid peroxidation, the procedure based on TBA described previously was adopted (Chung, 2006). The absorbance of the reaction mixture containing MDA–TBA adducts was measured at 535 nm.

**Statistical analysis.** Each experiment was carried out five times and each data point is expressed as mean ± SD (n = 5). The results were analysed using one-way analysis of variance and Tukey’s post hoc comparison with Prism (Version 3.02, GraphPad Software, Inc., San Diego, CA, USA).

### RESULTS

**Superoxide scavenging**

The results depicted in Figs 1A, 1B and 1C clearly show the superoxide scavenging activity of Zemaphyte® and its comparators, ascorbic acid and uric acid based on the nitroblue tetrazolium (NBT) reduction assay at 25 ºC. The pH of the test sample was adjusted to 7.4 where necessary to ensure the conditions were physiological and similar to the biological condition. The median inhibitory concentration 50% (IC50) of these samples indicate that Zemaphyte® (IC50, 0.0024% (wt/vol)) was about five times more efficient than uric acid (IC50, 0.0120% (wt/vol)), whilst it is approximately 10 times less potent than ascorbic acid (IC50, 0.00018% (wt/vol)) as a superoxide scavenger. A more useful comparison in terms of molar concentration could not be made as Zemaphyte® is a crude mixture.

To eliminate the possibility of false-positive results from the superoxide scavenging studies, the inhibitory effect of Zemaphyte® on the superoxide generation system, xanthine/xanthine oxidase was determined, by following the concomitant formation of the by-product, uric acid (Fig. 1D). Zemaphyte® did not affect the formation of uric acid (one-way analysis of variance; Tukey’s post hoc comparisons, p > 0.05), and hence, the generation of superoxide by the xanthine/xanthine oxidase system is unaffected, and this confirms the superoxide scavenging activity of Zemaphyte®.

**Interaction with hydrogen peroxide**

The results show that the hydrogen peroxide scavenging activity of Zemaphyte® is concentration dependent as measured using the scopoletin–HRP assay (Fig. 2A). At higher concentrations of Zemaphyte® (0.1–1.0%...
Figure 2. (A) Degradation of hydrogen peroxide (0.1 mM) (% apparent concentration of hydrogen peroxide) by Zemaphyte (0.01–1.00% (wt/vol)) at 20 °C as measured by the scopoletin–HRP method. (B) Degradation of hydrogen peroxide (0.1 mM) (% apparent concentration of hydrogen peroxide) by Zemaphyte (0.02 and 0.05% (wt/vol)) at time intervals (0–4 h) at 20 °C as measured by the scopoletin–HRP method. (C) Degradation of hydrogen peroxide (0.1 mM) (% apparent concentration of hydrogen peroxide) by Zemaphyte (0.01 and 0.05% (wt/vol)) with different concentrations of HRP (0.8–4.0 units/mL) at 20 °C as measured by the scopoletin–HRP method. Each data point is expressed as mean ± SD (n = 5).

Hydroxyl scavenging

The use of the deoxyribose assay to assess hydroxyl scavenging activity is usually comparable to the definitive technique of pulse radiolysis (Halliwell et al., 1987). The accuracy and reproducibility of the deoxyribose method are confirmed as the rate constants of ethanol (1.0–2.0 × 10^9 M^−1 S^−1; from five experiments) and mannitol (1.0–1.5 × 10^9 M^−1 S^−1; from five experiments) obtained here are consistent with the literature (ethanol, 1.2–1.4 × 10^9 M^−1 S^−1; mannitol, 1.3–1.4 × 10^9 M^−1 S^−1) (Chung, 2006; Halliwell et al., 1987), where the concentrations of ethanol and mannitol expressed in % (wt/vol) correspond to 0.5, 1, 2, 4, 6 and 8 mM (Figs 4C and 4D).

The results for hydroxyl scavenging show that Zemaphyte® possessed high activity especially at low concentrations (0.01–0.06% (wt/vol)) (Figs 4A and 4B). The reaction of Zemaphyte® with hydroxyl appeared to obey second order kinetics at low concentrations (0.01–0.06% (wt/vol)) with EDTA/ferric chloride/hydrogen peroxide as the hydroxyl generation system. The initial rates of the plots suggest that Zemaphyte® is probably a more efficient hydroxyl scavenger than mannitol, but less efficient than ethanol in terms of percentage weight (Figs 4C and 4D). Since Zemaphyte® contains a mixture of herbal extracts unlike ethanol and mannitol, the kinetics of the reaction could not be calculated.

The non-linear competition plots for Zemaphyte® suggest that there are interactions between Zemaphyte® and one or more components of the reaction mixture (Figs 4A and 4B). It is known, for example, that the ability of a scavenger to react with hydrogen peroxide may lead to a non-linear plot (Chung, 2006; Halliwell et al., 1987). It was shown that Zemaphyte® did not destroy hydrogen peroxide in the Clark’s oxygen electrode study (Figs 3A and 3B). Together with the data from the scopoletin–HRP assay (Figs 2A, 2B and 2C), the overall results suggest the complexity of Zemaphyte with hydrogen peroxide, and this could account for the
and its formation of MDA–TBA was significantly lower than the controls such as microsomes/ferrous sulfate/ascorbic acid and microsomes only (one-way analysis of variance; Tukey’s post hoc comparisons, \( p < 0.05 \)). The positive control, butylated hydroxytoluene (BHT) protected the microsomal membranes from peroxidation at all the concentrations tested (0.005–1.00% (wt/vol)); the level of protection was statistically similar to Zemaphyte (one-way analysis of variance; Tukey’s post hoc comparisons, \( p > 0.05 \)). At lower concentrations between 0 and 0.01% (wt/vol), BHT provided greater protection against lipid peroxidation compared with Zemaphyte, whilst between 0.1% and 1.0% (wt/vol)

**Figure 3.** (A) Representative curves showing oxygen formation by mixtures containing Zemaphyte (0.005–0.50% (wt/vol)) and 0.1 mM hydrogen peroxide in 50 mM KH2PO4–KOH (pH 7.4) after addition of catalase (200 units/mL, final concentration) at 20 °C as measured by Clark’s oxygen electrode. a, addition of Zemaphyte (0.005–0.50% (wt/vol)); b, addition of hydrogen peroxide (0.1 mM); c, addition of catalase (200 units/mL); d, addition of buffer. (B) % Oxygen formation by mixtures containing Zemaphyte (0.005–0.50% (wt/vol)) and 0.1 mM hydrogen peroxide in 50 mM KH2PO4–KOH (pH 7.4) after addition of catalase (200 units/mL, final concentration) at 20 °C as measured by Clark’s oxygen electrode. Each data point is expressed as mean ± SD (n = 5).

**Figure 4.** (A) Hydroxyl scavenging by Zemaphyte (up to 0.20% (wt/vol)) at 25 °C. (B) Hydroxyl scavenging by Zemaphyte (up to 0.060% (wt/vol)) at 25 °C. (C) Hydroxyl scavenging by mannitol (0.009–0.146% (wt/vol); equivalent to 0.5–8 mM) at 25 °C. (D) Hydroxyl scavenging by ethanol (0.0023–0.037% (wt/vol); equivalent to 0.5–8 mM) at 25 °C. The hydroxyl generation systems were ferric chloride/EDTA/hydrogen peroxide and ferric chloride/hydrogen peroxide. Each data point is expressed as mean ± SD (n = 5).

**Microsomal lipid peroxidation**

The formation of MDA–TBA indicated the occurrence of peroxidation in biological membranes. The results depicted in Fig. 5 show that Zemaphyte® decreased ferrous sulfate/ascorbic acid-induced microsomal lipid peroxidation at concentrations above 0.05% (wt/vol), and its formation of MDA–TBA was significantly lower than the controls such as microsomes/ferrous sulfate/ascorbic acid and microsomes only (one-way analysis of variance; Tukey’s post hoc comparisons, \( p < 0.05 \)). The positive control, butylated hydroxytoluene (BHT) protected the microsomal membranes from peroxidation at all the concentrations tested (0.005–1.00% (wt/vol)); the level of protection was statistically similar to Zemaphyte (one-way analysis of variance; Tukey’s post hoc comparisons, \( p > 0.05 \)). At lower concentrations between 0 and 0.01% (wt/vol), BHT provided greater protection against lipid peroxidation compared with Zemaphyte, whilst between 0.1% and 1.0% (wt/vol)
Zemaphyte appeared to be a more potent antioxidant. This indicates that Zemaphyte® is an effective inhibitor of lipid peroxidation although it is less potent than BHT at lower concentrations (up to 0.01% (wt/vol)).

**DISCUSSION**

This study has clearly shown that Zemaphyte® is an efficient antioxidant being capable of scavenging both superoxide and hydroxyl, and preventing peroxidation of biological membranes. The study on the interaction of Zemaphyte® with hydrogen peroxide using the scopoletin–HRP assay and Clark’s oxygen electrode described earlier gave contradictory results. The former indicates hydrogen peroxide is destroyed, whilst the latter clearly suggests Zemaphyte® does not interact with hydrogen peroxide. The failure of the scopoletin–HRP assay to detect hydrogen peroxide is clearly not due to inhibition of the enzyme, HRP. The overall results suggest Zemaphyte® probably forms a stable complex with hydrogen peroxide causing it to remain undetected by the scopoletin–HRP assay, but can be degraded by catalase to give oxygen and water as indicated by the Clark’s oxygen electrode experiment. Its *in vitro* antioxidant activity (superoxide and hydroxyl scavenging) is higher than some of the commonly encountered antioxidants such as mannitol and uric acid, whilst it is similar to butylated hydroxytoluene as a lipid peroxidation terminator.

Hydrogen peroxide is now believed to play a crucial role in signal transduction of T- and B-cells and other cell types (Los et al., 1995; Nakamura et al., 1997; Reth, 2002). Other reactive oxygen species (ROS) such as superoxide may also serve as second messengers for cell–cell signal transduction events (Reth, 2002). T-cell priming can be mediated by activated macrophages, and this process could be facilitated by hydrogen peroxide diffusing from macrophages to T-cells. The full activation of macrophages requires interaction with T-cells, therefore, if the production of hydrogen peroxide from macrophages is impaired, they may not be able fully to activate the T-cells and thus do not receive the signals for their own maturation (Reth, 2002). Moreover, T-cell receptor stimulation induces rapid hydrogen peroxide production and it has been suggested to act as a chemical mediator to facilitate cell signaling (Devadas et al., 2002; DeYulia et al., 2005).

Polyphenolic antioxidants, rutin and chlorogenic acid have been reported to reduce ROS levels in anti-IgE-activated mast cells and also to inhibit histamine release by these activated mast cells (Chen et al., 2000). These cells are implicated in the pathogenesis of atopic eczema, in a 2-phase T-cell dependent response. The first phase is characterized by a T-helper (Th) 2-like immune response with preferential production of Th2 cytokines, which stimulate IgE production, and stimulate eosinophil granulocytes and other inflammatory cells which contain skin- and tissue-damaging proteins. This is then followed by a switch to a second phase of expression of T-helper (Th) 1 cytokines, manifesting as atopic eczema. The switch of the initial Th2 to a Th1 response is probably regulated by cytokines produced by eosinophil granulocytes (Artik and Ruzicka, 2003; Rho et al., 2004). The implication of oxidative stress in atopic eczema is further exemplified by the ability of N-acetyl-l-cysteine, a precursor of glutathione to down-regulate interleukin (IL)-4, IL-5 and interferon (INF)-γ secretion in Th2, suggesting its potential therapeutic use in Th2 related diseases including atopic eczema (Bengtsson et al., 2001).

In summary, this study has clearly shown Zemaphyte® is an efficient antioxidant to scavenge both superoxide and hydroxyl, and prevents peroxidation of biological membranes. Therefore, it is conceivable that the reported down-regulation of immune pathways by Zemaphyte® in atopic eczema patients may be augmented by its antioxidant activity.

**REFERENCES**


