Review

Thuja occidentalis (Arbor vitae): A Review of its Pharmaceutical, Pharmacological and Clinical Properties

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Arbor vitae (*Thuja occidentalis* L.) is a native European tree widely used in homeopathy and evidence-based phytotherapy. Many reviews and monographs have been published on the herbal substance's description, mode of action and clinical use. However, no comprehensive evidence-based review is available. Therefore, our aim was to search MEDLINE databases and survey manufacturers for further details or unpublished data. This review presents the botany, ethnobotany and phytochemistry, especially the different contents of essential oil (Thujone) in relation to different extraction procedures of this medicinal plant. *Thuja*'s antiviral action and immunopharmacological potential, such as stimulatory and co-stimulatory effects on cytokine and antibody production and activation of macrophages and other immunocompetent cells, have been evaluated in numerous *in vitro* and *in vivo* investigations. Although no controlled trials have been conducted on *Thuja occ* alone, many clinical studies have been performed with a herbal medicinal product containing a special extract of *Thuja occ* and other immunostimulants, demonstrating its therapeutic efficacy and safety in respiratory tract infections.

Keywords: *Thuja occidentalis* – common cold – Thujone – Esberitox

Introduction

Thuja occidentalis, commonly known as Arbor vitae or white cedar, is indigenous to eastern North America and is grown in Europe as an ornamental tree (1). The plant was first identified as a remedy by native Indians in Canada during a 16th century expedition and was found to prove effective in the treatment of weakness from scurvy (2). In folk medicine, *Thuja occ* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism (3–6). Today, it is mainly used in homeopathy as mother tincture or dilution (7,8). In combination with other immunomodulating plants, such as *Echinacea purpurea, Echinacea pallida* and *Baptisia tinctoria*, this medicinal plant is also used as evidence-based phytotherapy for acute and chronic infections of the upper respiratory tract (9,10), and as an adjuvant to antibiotics in severe bacterial infections such as bronchitis, angina, pharyngitis, otitis media and sinusitis (11,12).

Several reviews and monographs describe the botany, constituents and some pharmacological properties, and the use of this herbal substance in the treatment of the common cold (3,13–17); however, the majority are old or written in German. Therefore, we aimed to compile an up-to-date, comprehensive and evidence-based review of *Thuja occ* that covers its botanical description, phytochemistry, *in vitro* and *in vivo* pharmacology, safety and efficacy. For this purpose, MEDLINE databases were searched and manufacturers of *Thuja*-containing preparations were contacted for further details or unpublished data.

Botanical Description

Thuja occidentalis was originally cultivated in North America. It is a native European tree with a maximal height of 15–20 m (Fig. 1). It has coniferous pyramidal features, with flattened branches and twigs in one plane, bearing small scale-like leaves (3). Over the whole year, the leaves are green, with the

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lower side showing a brighter green where resin glands also reside. Small, 1–2 cm long green to brown coniferous pins contain the seeds.

Ethnobotany

Previously, the genus *Thuja* (family: *Cupressaceae*) was considered to consist of the species *Thuja koraiensis* Nakai, *Thuja occidentalis, Thuja orientalis* L. and *Thuja plicata* D. Don (15,18) which are commonly cultivated in Central Europe.
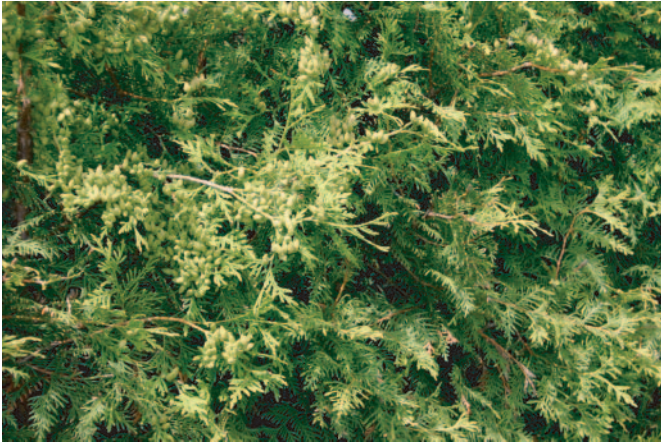

Figure 1. Typical twigs ad leaves of Arbor vitae (*Thuja occidentalis*). The figure shows the flattened branches and small coniferous pins containing the seeds.

However, more recent findings define the genus as follows: *T.koraiensis, T.occidentalis, T.plicata, Thuja standishii* (Gordon) Carr. and *Thuja sutchuenensis* Franch. Traditional names are American arborvitae, Arbor vitae, Arborvitae, Eastern white cedar, Hackmatack, Northern white cedar, Swamp cedar, *Thuja*, Tree of Life, white cedar and yellow cedar in English, and abendländischer Lebensbaum, amerikanischer Lebensbaum, Heckenthuja, Lebensbaum (German), Livsträ (Danish), Thuya d'occident (French), Thuia (Italian), Tuja (Russian), Livsträd (Swedish) and Zerav zapadni (Czech) (19).

Macroscopically, the cut herb shows small flattened, green twigs bearing paired, decussate leaves ~3 mm long. The keeled alternately lateral pairs partly conceal the flat rhomboidal, facial pairs pressed close together. The odour and taste are strongly camphoraceous (3). Only the dried twig tips of the species *Thuja occ* are used in the drug *Thujae occidentalis herba*. This consists of the varieties of *Thuja occ: Thuja occ* cv. *Aureospica, Thuja occ* cv. *Lutea, Thuja occ* cv. *Vervaeneana* and *Thuja occ* cv. *Wareana* (20). The cut herb should contain no more than 2% of the stem over a 4 mm diameter (Fig. 2). Table 1 presents the general description and distinguishing features of the four varieties.

Phytochemistry

The fresh plant (related to the dry substance) contains 0.6% essential oil, 2.07% reducing sugar, 4.9% water-soluble polysaccharides, 2.11% water-soluble minerals, 1.67% free
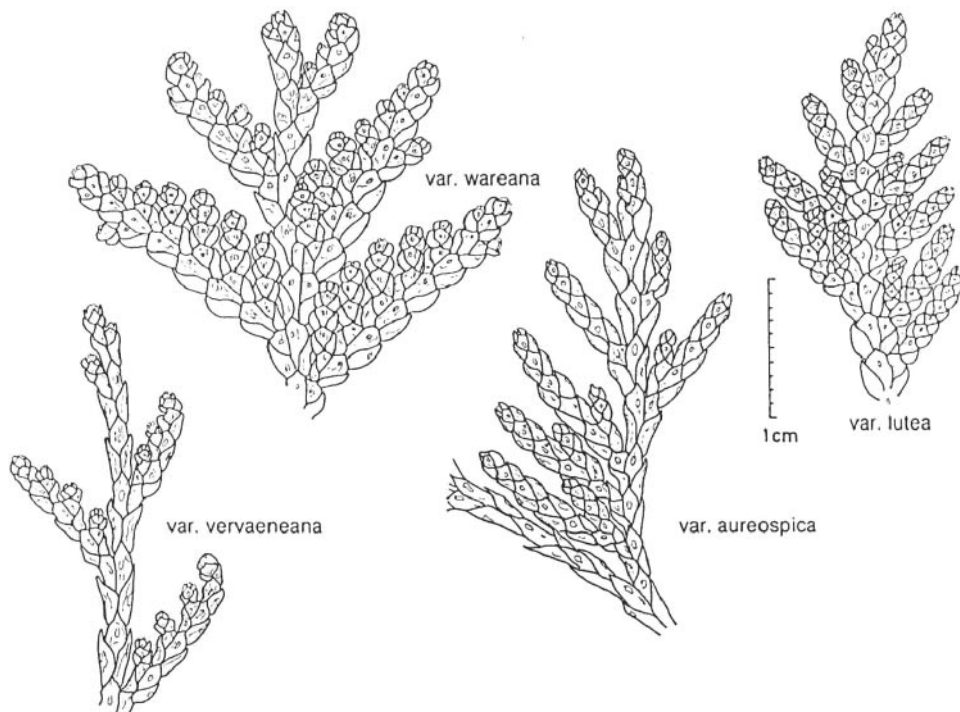
Figure 2. For the therapy, the dried herbal substance is used, which is called *Thujae occidentalis herba*. This figure shows details of the macroscopic appearance of the dried twig trips for the four variations *Thuja occ* cv. *Aureospica, Thuja occ* cv. *Lutea, Thuja occ* cv. *Vervaeneana* and *Thuja occ* cv. *Wareana*.

Table 1. The natural habitat of the herbal substance *Thujae occidentalis herba* including the four varieties *Thuja occ* cv. *Aureospica, Thuja occ* cv. Lutea, *Thuja occ* cv. *Vervaeneana* and *Thuja occ* cv. *Wareana*)

Variety	General description
Thuja occidentalis	Branchlets: the branchlets are flattened, dark green on the upper side and light-green or brownish on the base but without whitish markings. They all lie on the same plane, lateral sprouts of the last order with reference to the mother axis apical branching mostly to one side.
	Leaves: the leaves are differentiable into surface and marginal leaves. The visible leaf parts on the upper side of the branchlets are weakly convex; the under side of the branchlets is weakly concave. Surface leaves are characterized by a broad keel, greatly narrowing toward the tip. The tip is blunt to sharply pointed and slanted toward the sprout. Marginal leaves scaffold mostly pressed against the flanks of the surface leaves or the keels of the next higher marginal leaves. The marginal leaf margins are usually not in contact with the overlying surface leaf. They only run parallel to it for a short distance. Surface leaf tips extend beyond or just as far as the marginal leaf tips.
	Oil reservoirs: they are raised and lying on the keels of the surface leaves; present on the marginal leaves but not raised.
	Differentiating feature
Thuja occidentalis cv. *Aureospica*	Yellowish color, oil reservoirs less clearly raised, secondary leaf tips generally not extending beyond the marginal leaf tips, shinier.
Thuja occidentalis cv. *Lutea*	Broader sprouts (ratio L/B = 0.70), marginal leaf margins further apart, more densely branched, yellowish color.
Thuja occidentalis cv. *Vervaeneana*	'Joints' longer, surface leaf tips usually extend clearly beyond the marginal leaf tips (ratio: L/B = 1.66). Marginal leaf edges over surface leaves usually run parallel for a longer distance, oil glands often also raised on the marginal leaf keels. Cuticle thicker showing multiple cracks.
Thuja occidentalis cv. *Wareana*	Broader sprouts (ratio: L/B = 0.78), more densely branched, cuticle thicker and showing multiple cracks.

Table 2. The constituents of the dried herbal substance *Thujae occidentlis herba*

Group	Constituents
Essential oil (1.4–4% of drug)	Borneol
	Camphene
	Fenchone
	Limonene
	Myricene
	α-Terpine
	Terpinolene
	Thujone (0.76–2.4% of essential oil, 85% α-thujone, 15% β-thujone)
	Thujylalcohol
Coumarins	*p*-Coumaric acid
	Umbelliferone
Flavonoids	(+/−)-Catechine
	(−)-Gallocatechine
	Kaempherol
	Kaempherol-3-*O*-α-rhamnoside
	Mearnsitrin
	Myricetine
	Myricitrin
	Procyanidin B-3
	Prodelphinidin
	Quercetin
	Quercitrin
Other	Tannic acid (~1.3% of drug)
	Thuja polysaccharides and proteins (~4% of drug)
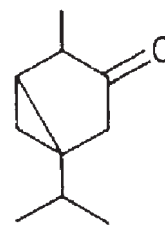
Figure 3. Thujone, which consists of 85% α-thujone and 15% β-thujone, is the major constituent in the essential oil from the dried herbal substance *Thujae occidentalis herba*.

acid and 1.31% tannic agents (14). The essential oil of the fresh leaves (related to the monoterpene fraction) contains 65% thujone, 8% isothujone, 8% fenchone, 5% sabines and 2% α-pinen as the main monoterpenes (14). Other monoterpenes, namely carvotanacetone, origanol, origanes, myrcen and camphen, have been described (14,21–23). Recently, further bioactive constituents have been found (1). High molecular weight glycoproteins/polysaccharides are highly relevant for the activity of the plant (16).

The constituents of the dried herbal substance *Thujae occidentalis herba* are listed in Table 2 (14,15,24–30). According to Hänsel *et al.* (15), the drug contains 1.4–4% essential oil, 60% of which is thujone, which corresponds to 2.4% thujone in the whole drug. Thujone occurs in nature as a mixture of α- and β-isomers (31,32). According to the European Agency for the Evaluation of Medicinal Products (EMEA), the content

of thujone in dried twigs was determined as 7.6 mg/g, consisting of 85% α-thujone and 15% β-thujone (Fig. 3). The equilibrium mixture consists of 33% α-thujone and 67% β-thujone (27).

A critical factor for *Thuja*'s use as a medicinal herb is its content of essential oil. Indeed, the content of thujone seems to be significantly affected by different extraction procedures. The highest content of essential oil was found in extracts obtained by distillation, whilst percolation with purified water reduced the thujone content in the extract to the lowest level (29). Using purified water as a solvent, an average of 0.6 mg of thujone was extracted from 1 g of drug during percolation
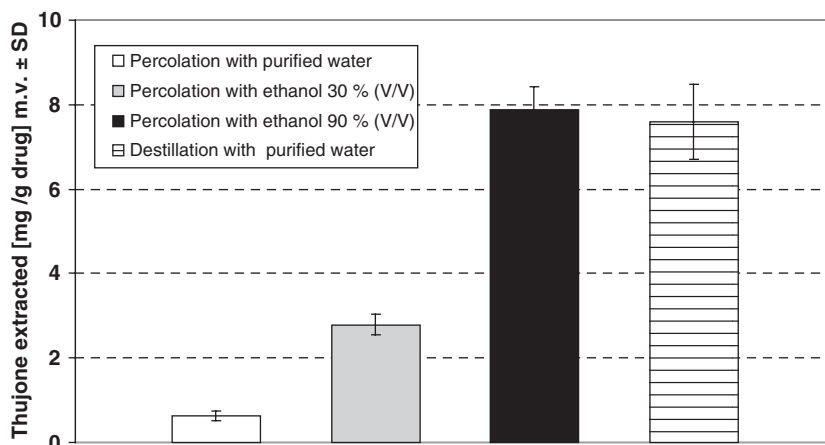
Figure 4. The solvent for extraction significantly influences the amount of thujone in the extracts of the dried herbal substance *Thujae occidentalis herba*.

(30). In contrast, when 30% (v/v) ethanol was used, 2.8 mg of thujone was extracted from 1 g of *Thujae occidentalis herba*, and >2.5-fold higher amounts of thujone (7.9 mg) were attained with high ethanol concentration (90% v/v) (30). From the result of 12 batches, the mean value for the thujone content was 1.16 mg/g of herbal substance and the maximum value was 2.95 mg/g, corresponding to 0.30%. Using a special extraction procedure with 30% (v/v) ethanol, the content of thujone in the extract can be reduced to one-third of the amount obtained by extraction with ethanol 90% (v/v) (Fig. 4).

Pharmacological Properties

The immunopharmacological potential of *Thuja* has been investigated in various *in vitro* and *in vivo* test models (33–35). Of the many existing studies on this subject, only the most relevant *in vitro* and *in vivo* investigations will be described in greater detail in the following.

In Vitro Studies

HIV-1 Activity

Thuja polysaccharides (TPS) inhibited human immunonodeficiency virus (HIV)-dependent cell death at a final concentration of 625 µg/ml. At this concentration, TPSg was shown to be completely non-toxic for MT-4 cells, which had not been infected with HIV-1. TPS were shown to inhibit HIV-1-specific antigen expression on freshly infected MT-2 cells in a dose-dependent manner (36,37).

Spleen Cell Proliferation

The *Thuja* retentate fraction (obtained by dialysis of an aqueous-ethanolic extract of *Thuja occ* cut-off: 10 000 Dan, Amicon SP20, S10Y10) caused an increase in the proliferation rates of spleen cells from NMRI and C3H/HeJ mice. In comparison with the medium control, a significantly higher incorporation rate was found up to a concentration of

7.8 µg/ml. The maximum stimulation indices were 5.3 in the tests with spleen cells from NMRI mice. In the tests with C3H/HeJ spleen cells, stimulation index (SI_{max}) values of 3.4 were reached (38).

CD4-positive T Cells

Polysaccharide fractions with mol. wts ranging from 20 000 to >1 000 000 Da were isolated from the aqueous alkaline extract of the herbal parts of *Thuja occ* by ethanol precipitation and fractionation by ultrafiltration. High molecular weight subfractions of TPS proved to be highly mitogenic on peripheral blood leukocytes. It was demonstrated that the mitogenic and cluster-forming activity of TPS causes T-cell induction particularly of the CD4-positive T-helper/inducer cells in connection with an increased production of interleukin-2 (IL-2). This indicates that not only proliferation but also a differentiation to fully functional T-helper cells takes place. Only T cells, not B cells, were stimulated by TPS. The activation occurred only in the presence of the monocyte/macrophage fraction and was neutralized by antibodies against interferon (IFN)-γ and IL-1. TPS-induced IFN-γ production in $CD4^+$ cells was assumed to be the mode of action, whereby the IFN-γ produced stimulated monocytes/macrophages to produce IL-1. As a second signal of the T-cell activation, this triggered the expression of IL-2 receptors and the production of IL-2 in $CD4^+$ T-helper cells (39,40).

Cytokine Induction

The incubation of P388C mouse macrophages with 10–100 µg/ml of the retentate fraction from *Thujae occidentalis herba* led to an increased secretion of the cytokines IL-1, IL-6 and tumor necrosis factor-α (TNF-α) in the cell culture supernatant. With regard to TNF-α, the retentate fraction from *Thuja* showed concentration-dependent activity (38).

These results were confirmed in cell culture assays with human blood monocytes, where incubation with the *Thuja* retentate fraction also induced a significant increase in IL-1,

IL-6 and TNF-α secretion. The cytokine titers in the supernatants were higher than the titers induced by phytohemagglutinin (PHA; 10 μg/ml).

After incubation of NMRI spleen cells with the *Thuja* retentate fraction, an increase in the production of IFN α_β was observed. After application of the *Thuja* retentate, a maximum of 19 U/ml IFN α_β was determined in the cell culture supernatants, whereas no IFN α_β activity was detected in the supernatants from the medium control. Activity was also shown for the positive control substance concanavalin A which induced an IFN α_β production of 19 U/ml at a concentration of 2 μg/ml.

Antibody Production

The retentate fraction produced a concentration-dependent increase in the number of antibody-producing lymphocytes in the hemolytic plaque assay *in vitro*. The number of anti-SRBC-(sheep red blood cell)-IgM-producing plasma cells rose, as did all Ig-secreting plasma cells, as registered by the 'reverse' technique using protein A-labeled SRBCs. Incubation with lipopolysaccharide (LPS) as positive control also led to a concentration-dependent increase in the number of plaque-forming cells (41).

NO_2^- Production

The influence of *Thujae occidentalis herba* on the oxidative metabolism of macrophages was determined by measuring the NO_2^- production of mouse alveolar macrophages (MH-S) after 48 h incubation with the respective test fractions. The NO_2^- production of the MH-S cells was increased by incubation with the retentate fraction in a concentration-dependent fashion from 2 ± 0.3 μmol/l (medium control) to a maximum of 42.5 ± 2.7 μmol/l. The combined addition of IFN and LPS (1 + 5 ng/ml) served as a positive control in this test system (Fig. 5) (38).

The results of the *in vitro* investigations indicate an immunomodulatory and antiviral potential of *Thuja occ.* The main target of the immunologically active constituents seems to be the macrophage/monocyte fraction. The activation of macrophages manifested itself in increased cytokine secretion and the stimulation of NO_2^- production. The presence of macrophages was a prerequisite for the activation of CD4-positive T cells, thus indicating a decisive role for macrophages also for the observed effects in spleen cell cultures i.e. stimulation of cell proliferation and antibody production.

In Vivo Studies

Leukocyte Counts in Mice

A single dose of *Thuja* extract (i.v.) led to a dose-dependent left shift in white blood counts within 4 h. The segmented and stab granulocytes in the blood of the animals receiving the extract increased 3- to 4-fold compared with the control animals. The percentage of segmented granulocytes increased from 9 to 36%, and the percentage of stab granulocytes from 3 to 15%. Correspondingly, the number of lymphocytes decreased in comparison with the control mice (from 89 to 48%). The effects were transitory, i.e. the blood count had normalized after 15 h at the latest (38).

Cytokine Induction

The i.v. administration of a retentate fraction of *Thujae occidentalis herba* induced a significant increase in TNF-α, IL-6 and IL-1 activity in the serum of treated mice compared with control mice that had received physiological NaCl only. The only dose dependency was found with regard to the induction of IL-6 and IL-1 (38).

Stimulation of cytokine production could also be seen in irradiated mice after i.v. application of *Thuja* TPSg fraction. CBF$_1$ female mice were irradiated with 3 or 6 Gy at a dose rate of 17 cGy/min. A total of 144 mice were given an i.v. injection of TPSg. Forty-eight control mice were injected with RPMI-1640 medium alone. At 4–15 days after TPSg injection, the mice were sacrificed and tested for the number of c.f.u.-GM
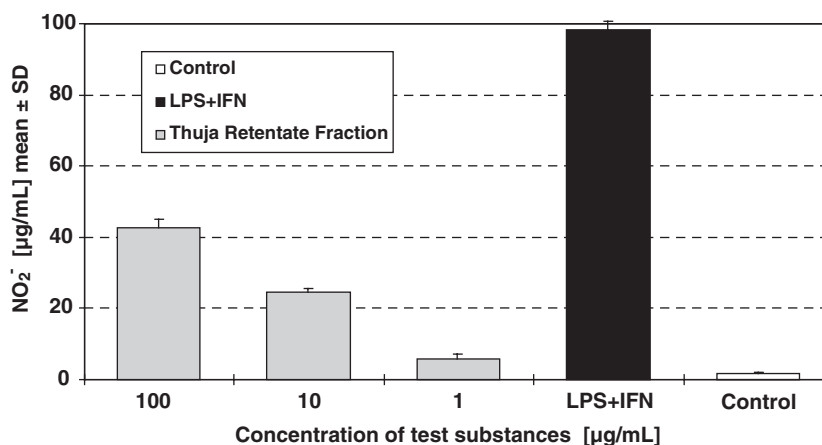
Figure 5. Increase in the NO$_2$ production of alveolar macrophages through the retentate fraction from *Thuja occidentalis herba* (100, 10 and 1 μg/ml). Positive control substance: LPS (5 ng/ml) + IFN-γ (1 ng/ml). Data are expressed as the mean value ± SD (*n* = 4).

and c.f.u.-S from both bone marrow and spleen. Seven days after i.v. injection of 2.5 mg of TPSg per mouse, the titers of c.f.u.-GM, c.f.u.-S-8 and c.f.u.-S-11 in the bone marrow and spleen of 3- or 6 Gy-irradiated and TPSg-treated mice were significantly increased ($P < 0.01$) compared with irradiated mice not receiving TPSg (42).

After oral application of an aqueous-ethanolic extract of a mixture of *Thujae occidentalis herba, Baptisiae tinctoriae radix, Echinaceae purpureae radix* and *Echinaceae pallidae radix*, there were no recognizable differences in the cytokine titers [IL-2, IFN-γ, granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-1 and TNF-α] between sera of NMRI mice that had been treated perorally with the herbal extract for a period of 1–9 days and the sera of the control mice. Concentrations of cytokines in sera of both groups ranged around the detection limit of the respective assays. Spleen cells from NMRI mice, however, that had been treated with the extract for 1–9 days produced higher amounts of IL-2, GM-CSF and IFN-γ *ex vivo* in comparison with those from the control mice especially after additional stimulation by LPS or concanavalin A *in vitro*. Application of the extract also triggered the production of IL-1 and TNF-α by peritoneal macrophages *ex vivo*, particularly after co-stimulation with LPS *ex vivo*, thus mimicking the action of microbial antigens, such as bacterial LPS, in the case of infections (43).

Thus the peroral administration of the *Thuja*-containing preparation did not induce any systemic increase in cytokine titers, but probably caused a local activation of cytokine-producing cells for 'priming'. Thus, after local contact with the immunomodulatory active components of the extract in the gut or in the upper part of the bronchial system, cells are able to react to an additional stimulation causing increased cytokine secretion. Under physiological conditions, such a local modulatory cytokine induction is to be assessed more positively than a systemic one, which in the case of an overexpression could cause considerable side effects (43).

Antibody Response in Normal and Immunosuppressed Mice

Oral application of the extract significantly enhanced antibody response to SRBCs in healthy NMRI mice, inducing an increase in numbers of splenic plaque-forming cells (PFCs) and titers of specific antibodies in sera from treated mice. In mice immunosuppressed by old age or additional treatment with hydrocortisone, treatment with the extract resulted in a normalization of antibody response to SRBCs.

To prove the hypothesis of a gut-associated lympho-epithelial tissue (GALT) activation, the effects of *Thuja* on immunoreactivity of Peyer's patches (PP) cells was investigated by measuring the anti-SRBC antibody response of PP cells *ex vivo* using the hemolytic plaque technique. For this purpose, a *Thuja* retentate fraction was administered by gavage to five mice at a dose of 1 mg. Mice in the control group received a corresponding amount of matrix without test substance. After 15 h, mice were sacrificed and the PP cells were isolated and incubated with medium, LPS or the *Thuja* retentate

fraction on cell culture plates. The number of antibody-producing cells was determined after 7 days using the reverse hemolytic plaque technique. The extract significantly enhances antibody response to SRBCs in PP, thus indicating that immunologically active macromolecules can contact cells of GALT and modulate the mucosal immune response (43,44).

Immune Response in Mice after Long-term Treatment

The effects of an orally administered aqueous-ethanolic extract of a mixture of *Thujae occidentalis herba, Baptisiae tinctoriae radix, Echinaceae purpureae radix* and *Echinaceae pallidae radix* on the immune response in mouse were investigated over the short and long term. In all investigated mouse strains, the extract induced an increase in the numbers of PFCs and in the titers of specific antibodies in the sera of treated mice. Administration of the extract over several months also stimulated the PFC response without affecting spleen weight, total cell yield per spleen or white blood cell count (41).

Influenza Virus Type A Infection in Mice

The effects of oral application of an aqueous-ethanolic extract of a mixture of *Thujae occidentalis herba, Baptisiae tinctoriae radix, Echinaceae purpureae radix* and *Echinaceae pallidae radix* on the course of influenza A virus infection was investigated in Balb/c mice. The mixture extract was administered to mice via their drinking water for 14 days, starting 6 days before intranasal infection with influenza A virus. The extract induced a statistically significant increase in survival rates, prolonged mean survival time and reduced lung consolidation and virus titers. The experiment demonstrated that the plant immunomodulator given 6 days before exposure is a potent inhibitor of influenza A virus pathology *in vivo*.

Because of the proven immunomodulatory efficacy of the preparation, it was assumed that the positive effect of this extract exerted on infected mice is mediated first of all by its immunostimulating activity and that the direct antiviral activity is rather of secondary importance (45).

Clinical Efficacy

While there are many pre-clinical investigations in the literature, no data on clinical trials using *Thuja occ* as a single herbal substance are available. In contrast, many clinical studies have been conducted with a herbal medicinal product containing an aqueous-ethanolic extract of a mixture of *Echinacea purpurea, Echinacea pallida, Baptisia tinctoria* and *Thuja occ* [Esberitox®, Schaper & Brümmer, Salzgitter, Germany (33,34)]. In most of these studies, the therapeutic efficacy of the *Thuja*-containing herbal product was proven in the treatment of acute respiratory tract infections and the common cold. Two of these pre-GCP (Good Clinical Practice) clinical trials showing good therapeutic efficacy of the herbal product on viral upper respiratory tract infections (URIs) are particularly noteworthy. One randomized, placebo-controlled, double-blind study enrolled 100 subjects (10). After just 3 days, the patients from the active

treatment group showed significant improvements in their common cold symptoms. In the other randomized, placebo-controlled, double-blind study (9), 150 patients with URIs were treated with either Esberitox or placebo. In this latter study, Esberitox showed a similar efficacy result on URI symptoms to that in the former study. In addition, a significant enhancement of the immune response was observed in recall antigens. In four other studies, the herbal product was also found to be beneficial when used as an adjuvant to antibiotic treatment of bacteria-induced URI (11,12,46,47). In the following section, only the more recent and GCP-compliant studies are discussed in detail.

Common Cold

In a double-blind, placebo-controlled multicenter study (15 practitioners in Germany), patients suffering from acute common cold were randomized to receive Esberitox (*Thuja* daily dose corresponding to 6 mg three times daily (t.i.d.)] or placebo for 7–9 days (48). On a daily basis, the patients documented the intensity of 18 cold symptoms and their overall cold status on a 10-point scale and rated their general well-being using Welzel–Kohnen color scales. Additionally, a physician assessed the severity of illness on days 4 and 8 using the Clinical Global Impression (CGI-1). The main and confirmatory outcome measure was expressed as a total efficacy value. This was gauged from the z-standardized area under the curve (AUC) values of the primary end-points (rhinitis score, bronchitis score, CGI-1 and general well-being). Adverse events, overall tolerability, vital signs and laboratory parameters were documented. A total of 259 patients were eligible to be evaluated (intention to treat; ITT). The primary efficacy parameters demonstrated the herbal product's superiority over placebo ($P < 0.05$). The effect size was 20.6% of the SD [90% confidence interval (CI) 0.04–41.1] in the ITT population and 23.1% (1.7–44.5) in the valid case population. In relation to general well-being, the effect size was 33.9% of the SD (12.5–55.3%, VC). The efficacy of the drug was most prominent in the subgroup of patients who started therapy at an early stage of their cold or with a symptom score of ≥4 (Fig. 6). In the drug group, patients suffering symptoms of at least moderate intensity at baseline showed a response rate (at least 50% improvement of the global score, day 5) of 55.3%, and those in the placebo group showed a response rate of 27.3% [$P = 0.017$; number needed to treat (NNT) = 3.5]. This study showed the therapeutic benefit and superiority of Esberitox versus placebo for decreasing the duration of the common cold and alleviating symptoms. A greater benefit can be expected if patients with the common cold are able to start taking the drug as early as practicable after manifestation of initial symptoms.

In another double-blind, placebo-controlled study, 94 adults (age >18 years) suffering from the common cold for no longer than 1 day were randomized to two different treatment groups: active substance (the *Thuja* daily dose corresponding to 6 and 12 mg t.i.d) or placebo (49). The results of the primary efficacy criteria for evaluation (reduction of total number of tissues used throughout the entire observational period) were confirmed to be statistically significant and clinically relevant. This was true for both the ITT analysis ($P = 0.0259$) and the per protocol analysis ($P = 0.0146$). Subsequent pairwise group comparisons showed a statistically significant superiority of the high dose compared with placebo ($P = 0.0323$). Similar results were obtained for the secondary efficacy parameters such as the number of tissues used on day 2 ($P = 0.0071$) and day 5 ($P = 0.0495$). From these results, it can be concluded that the clinical efficacy of the *Thuja*-containing herbal medicinal product has been confirmed for the treatment of acute common cold.

Supportive Therapy to Standard Antibiotics

Many antibiotics are known to have a suppressive effect on the immune system. A total of 53 patients scheduled to receive antibiotics for treatment of acute exacerbation of chronic bronchitis (as an example of a severe bacterial infection requiring antibiotics) were enrolled in a prospective, multicenter,
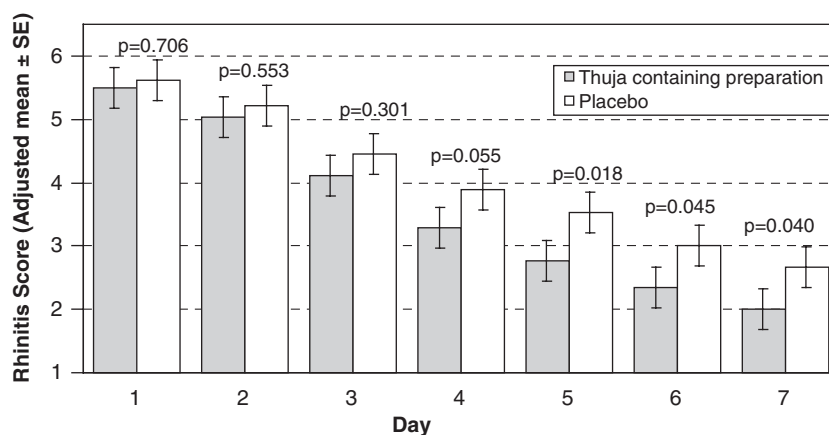
Figure 6. Improvement of rhinitis scores (congested nose, runny nose, sniffling, frequency of handkerchief use, frequency of sneezing). Mean symptom scores (baseline adjusted mean and SE) are shown for placebo ($n = 57$) and the *Thuja*-containing herbal remedy ($n = 58$). *P*-values for the differences between treatment groups are given for the patients with a symptom score of ≥4 at day 1 (repeated measures; co-variates at day 1).

double-blind, placebo-controlled study (50). The aim of this research was to confirm any beneficial effects of the co-medication of antibiotics with the drug in subjects with acute exacerbation of chronic bronchitis. The main inclusion criteria were chronic bronchitis, staged to forced expiratory volume of the first second (FEV_1) with a value between 35 and 75%. Patients were randomly assigned to receive antibiotics plus either the herbal product (Esberitox, $n = 25$; *Thuja* daily dose corresponding to 12 mg t.i.d.) or placebo ($n = 27$). Antibiotic therapy was administered according to generally accepted guidelines and the study medication was given concomitantly for 28 days. The baseline-adjusted means for FEV_1 (%) on day 10 were 68.7 points for the drug group and 59.2 points for the placebo group ($P = 0.0303$), showing a faster improvement in the drug group. For FEV_1, the difference between the two treatment groups was 267 ml ($P = 0.0499$). The time to half-maximal improvement was 5.7 days in the drug group compared with 12.8 days in the placebo group.

Clinical Safety

Symptoms of intoxication from the fresh *Thuja* plant include vomiting, stomach ache, diarrhea and gastroenteritis followed by absorption disorders, headache, nervous agitation and chronic convulsions, and symptoms of liver and renal toxicity extending to yellow liver atrophy, arrhythmia and myocardial bleeding (51). In cases of overdose and abuse, the oral intake of *Thuja* extracts induced severe metabolic disturbances. Intoxication was accompanied by an irritant effect on the gastrointestinal tract, uterus, liver and kidney. Infants who ingested leaves and twigs of fresh plant showed mild gastrointestinal disorders and vomiting (24).

These reactions can be explained by the high contents of thujone in the fresh plant. This ingredient occurs in significant quantities in the essential oil fraction *Thuja occ* (see 'Phytochemistry' above) and many other plants that are part of the human diet such as *Salvia folium*, widely used as spice and in teas, or *Artemisia pontica*, for the production of vermouth wine (52). Thujone is reported to be the toxic agent in absinthe, a liqueur popular in the 19th and early 20th centuries, and to have antinociceptive, insecticidal and anthelmintic activity (53). The acute toxicity information available for thujone was reported as follows (LD_{50} in mg/kg): rats oral, 500; rabbit i.v., 0.031; rabbits dermal, 5000. The lethal dose appeared at 0.2 ml/kg body weight (BW) (54). In other investigations, porphyrogenic properties or mitogenic activities were reported (55,56). The Committee on Veterinary Medicinal Products noted that the LD_{50} values stated for the constituent thujone were 87.5 mg/kg of BW after subcutaneous administration in mice and 240 mg/kg BW after intraperitoneal administration to rats (24). In an *in vitro* investigation, however, thujone was demonstrated to be non-toxic (57) and an oral dose of up to 1.25 mg thujone/kg BW is considered harmless in humans (58). Up to a single daily dose of 75 mg is reported to be safe in humans. In their opinion issued on 2 December 2002, the Scientific Committee of the European Commission stated that a 60 kg adult who consumes one litre of an alcoholic beverage containing 5 mg/l thujone, i.e. the maximum permitted level of thujone in alcoholic beverages with up to 25% alcohol, would ingest ~0.08 mg thujone/kg BW. This intake is ~100 times lower than the no effect level (NOEL) derived from a 14 week study in rats (59).

Adverse Events in Clinical Trials

The safety of *Thuja*-containing preparations especially those extracts percolated with ethanol 30% (v/v) can be regarded as good. In a randomized, double-blind, placebo-controlled study, 26 patients receiving *Thuja* and 23 patients receiving placebo showed adverse events (48). Adverse drug reactions (ADRs) were suspected in five patients in the placebo group and in two patients in the active treatment group [one adverse event in the drug group (sleeplessness) was deemed possibly related to the study medication]. No serious adverse event occurred. In another study, 19 mild to moderate adverse events (drug = 8, placebo = 11) in 14 subjects were documented (50). None of them was assessed as serious and no event was classified as an ADR. In a recent double-blind study, no adverse events were reported (49). The investigators concluded that the herbal product can be recommended as a safe treatment for acute viral respiratory tract infections. In addition, no adverse events were reported in another study (60). One study (9) showed that three patients of both study groups experienced mild nausea. Additionally, one patient treated with the Thuja-containing preparation complained of rheumatic symptoms and one patient from the placebo group reported heartburn. All adverse events were classified as 'mild' to 'moderate' and judged to be unrelated to the study medications.

Pregnancy and Lactation

Thujone-containing plants and preparations do not have direct or indirect abortive effects (52). Like many other herbs, decoctions of *Thuja* have traditionally been used for its abortive properties, presumably mixed with Savin tops (15). Such trials have been carried out repeatedly by laymen, but have failed nearly every time to prove any abortive properties and have often resulting in lethal toxicity. Deadly apnea can occur if large quantities of thujone are absorbed by the circulatory system (14). Over the many years of experience with Esberitox, no such safety concerns have been reported. For medical practice, however, it is recommended that *Thuja* not be used during pregnancy and lactation before consulting a doctor.

Post-marketing Data

Our search shows that *Thuja* is only used phytotherapeutically in the product Esberitox, a herbal medicinal product that has been marketed throughout the world for many decades. Considering the last 5 years of marketing (January 1999–August 2003), the distribution of the number of sold and/or prescribed packages of the herbal product corresponds to a total amount of 1150 million units (1 unit corresponds to

2 mg of *Thuja*). Assuming an average of 7–14 days of treatment with the maximum dosage (18–36 mg *Thuja*) as directed, this corresponds to 12.2 million treated patients in the covered period worldwide. During these periods, a total of 63 ADR reports with Esberitox were recorded. The majority of the ADRs were of a mild and transient nature. Classified by organ systems, skin and appendage disorders, such as skin rashes, were reported most frequently. This corresponds to an incidence of about five ADRs per 1 million treated patients. During this period, only one case of a serious and unexpected ADR of acute sarcoidosis (Löfgren's syndrome) was reported in the literature (61). Since the woman had injected a mistletoe preparation in addition to Esberitox, with a positive lymphocyte transformation test (LTT), a causal relationship with the *Thuja*-containing herbal product was assessed as 'unlikely'.

The recommended daily dose of Esberitox in adults is a dose corresponding to 18–36 mg of *Thuja*. Children should take 12–24 mg. This is equivalent to a daily dose of 18–36 µg of thujone for adults and 12–24 µg for children. This daily dose is several orders of magnitude below the threshold of 1.25 mg thujone/kg BW considered harmless for humans, and also below the maximum level of thujone permitted in alcoholic beverages (~0.08 mg thujone/kg BW for an adult).

Summary and Conclusion

Thuja occidentalis is widely used in homeopathy and evidence-based phytotherapy. Its immunopharmacological potential has been demonstrated in numerous *in vitro* and *in vivo* test models showing its immunostimulating and antiviral activities. A critical factor for *Thuja*'s use as a medicinal herb is its content of thujone, which is reported to be the toxic agent of many fresh plants that are part of the human diet. However, the content of thujone in *Thuja*-containing finished products depends on the extraction procedure; thus it was shown that percolation with ethanol 30% (v/v) can significantly reduce the thujone content in comparison with the percolation by using ethanol 90% (v/v) for extraction. Three GCP-compliant, double-blind, placebo-controlled clinical studies have verified the efficacy and safety of a herbal medicinal product containing *Thuja* (Esberitox) for treatment of the common cold, and its efficacy as an adjuvant to standard antibiotic treatment of severe bacterial infections. In these studies, only mild to moderate adverse events were documented with a rare frequency, and none were assessed as serious. Based on the recommended daily dose and the manufacturing procedure, the amounts of thujone that would be ingested are well below the threshold limit values considered harmless for humans, and even below the maximum permitted level in alcoholic beverages. Over the past 5 years, >12 million patients have been treated with this herbal medicinal product worldwide. During the same period, ADRs of a mild to moderate nature were reported with a rare frequency, but none was judged to be relevant to the safety of the herbal product. Thus, it can be concluded that due to a special extraction procedure, this *Thuja*-containing preparation is a safe and effective herbal medicinal product for treatment of the common

cold. The recommended daily dose of up to 36 mg of *Thuja* is within a safe and non-critical range. Notwithstanding these positive data, further comprehensive evidence from clinical trials, especially with the herbal substance alone, is still needed.

Conflict of Interest:

The authors Dr C. Bodinet, Dr B. Naser and Dr M. Tegtmeier are all employed by the company Schaper & Brümmer which produces the Thuja containing preparation. Dr U. Lindequist declares no conflict of interest.

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