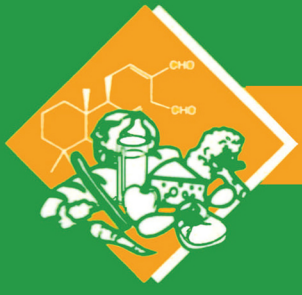
Herbs, Botanicals, and Teas

EDITED BY

G. Mazza
B.D. Oomah



FUNCTIONAL FOODS AND NUTRACEUTICALS SERIES

CRC Press
Taylor & Francis Group

Herbs, Botanicals, and Teas

FUNCTIONAL FOODS AND NUTRACEUTICALS SERIES

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Functional Foods: Biochemical and Processing Aspects
Volume 1
Edited by G. Mazza, Ph.D.

Herbs, Botanicals and Teas
Edited by G. Mazza, Ph.D., and B.D. Oomah, Ph.D.

Functional Foods: Biochemical and Processing Aspects
Volume 2
Edited by John Shi, Ph.D., G. Mazza, Ph.D., and Marc Le Maguer, Ph.D.

Methods of Analysis for Functional Foods and Nutraceuticals
Edited by W. Jeffrey Hurst, Ph.D.

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

Originally published 1998 by Technomic Publishing

Published 1998 by CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

ISBN-13: 978-1-56676-851-1 (hbk)

Library of Congress Cataloging-in-Publication Data

Main Entry under title:
Functional Foods & Neutraceuticals Series: Herbs, Botanicals and Teas

Library of Congress Card Number 00-102584

Table of Contents

Series Editor's Preface

THE Functional Foods and Nutraceuticals Series was developed to serve all those involved with and interested in foods and/or food components that provide health benefits beyond those that can be attributed to basic nutritional functions. It offers a comprehensive treatment of the emerging science and technology of functional foods and nutraceuticals shown to play a role in preventing or delaying the onset of diseases, especially chronic diseases. Books in the Series cover a wide range of developments in chemistry, biochemistry, pharmacology, epidemiology and engineering of products from plant and animal sources, results of animal and clinical trials and regulatory, standardization and quality control issues. At least one volume will deal with methods of analysis of functional foods and nutraceuticals.

In developing the series, the Series Editor and Technomic Publishing Company, Inc., recognized the need for assembling, reviewing, condensing and disseminating the rapidly accumulating information on food and health issues to food scientists and technologists, nutritionists, public health professionals, regulators, entrepreneurs and sophisticated consumers. The Series was launched at a time when the diet-health paradigm of food being the source of essential nutrients to sustain life and growth was beginning to change into one in which foods are also called on to provide protection against diseases. The paradigm shift is occurring as consumer interest in diet and health is at an all-time high, and it is due in part to the growing body of epidemiological evidence associating a diet rich in fruits and vegetables with the reduced risk of certain types of cancer and other chronic diseases.

Health professionals are gradually recognizing the role of phytochemicals in health enhancement, and currently there is a frenzy of epidemiological

animal and human studies. Emerging results indicate that specific phytochemicals may prevent chronic diseases, aid in the management of symptoms of chronic disorders, improve immune response and reduce negative effects of aging. Food components such as anthocyanins, catechins, lycopene, lutein, allicin, isoflavones, resveratrol, lignans, isothiocyanates, phytosterols, omega-3 polyunsaturated fatty acids, saponins and complex polysaccharides have been shown to modify metabolic processes and influence disease risks. Elucidating the chemistry, biology, pharmacology and the many potential health effects of the countless bioactives present in plant and animal products, developing sound scientific data to support health claims and developing food products that address the needs of an increasingly health conscious consumer interested in self-medication offer tremendous challenges and opportunities for the scientific community and the food industry worldwide.

The aim of this book series is to bring together, in a timely manner, reliable information that will serve the needs of food, nutrition and health practitioners, provide reference to material that may otherwise be difficult to locate and provide a starting point for further research.

G. Mazza
Series Editor

Preface

CONSUMERS, especially baby boomers, who have grown disillusioned with modern medicine, pharmaceuticals and the healthcare system and who want more control over their health, are driving the ever-growing demand for functional foods and nutraceuticals. As a result, there has been an explosion of interest in information on herbs, botanicals and teas in recent years. It has become clear that a book on the current state of knowledge of these products is needed. *Herbs, Botanicals and Teas,* written by leading researchers presently contributing to this field, provides the latest scientific and technical information on the chemical, pharmacological, epidemiological and clinical aspects of garlic, ginseng, Echinacea, ginger, fenugreek, St. John's wort, Ginkgo biloba, goldenseal, saw palmetto, valerian, evening primrose, licorice, bilberries and blueberries, and green and black teas. The book contains 13 chapters, 10 on herbs and botanicals, one on teas, one on international regulations and the last chapter on quality assurance and control for the herbal and tea industry.

The content of a typical chapter on a given product includes an introduction, sections on chemical composition and chemistry, physiological actions of the product, clinical or therapeutic applications, conclusions, and references. Whenever possible, results of chemical, biological and/or clinical studies are presented in tables or figures. The chapter on ginseng, for example, presents and discusses the latest research results on its chemical and pharmacological properties. Physiological actions addressed include endocrine activity, neurotransmitter activity, neurophysiological responses, antioxidant activity and ginseng's effects on the immune system. Similarly, the chapter on evening primrose reviews the current knowledge of evening primrose oil (EPO) and its major constituent gamma-linolenic acid (GLA). It also contains an in-

depth discussion of the result of recent research on the metabolism of GLA, nutritional relevance of EPO, the role of EPO as a functional food and therapeutic applications of EPO in cardiovascular disease, diabetic neuropathy, gastrointestinal, gynecological, and neurological disorders, rheumatoid arthritis, viral infection and immunological disorders.

The distinction between herbs, botanicals and teas may not always be apparent in this book. The reason for this seeming lack of clarity is that these terms are defined in several ways depending on the context in which the words are used. The term herbs generally refers to plants with leaves or stems that are used for medicine, seasoning, food or perfume. Here it is defined as a crude extract, or a product, produced from an edible plant and sold in pill, powder and other medicinal forms and demonstrated to have a physiological benefit or provide protection against disease. A botanical is traditionally a drug made from roots, leaves, flowers or other parts of plants. It can also imply herbal preparation, herbal tea, herbal mixture or medicinal herb. Here it is synonymous with nutraceutical. The term teas refers to black and green teas made from the leaves of *Theae nigrae folium.*

With over 2,000 scientific references, this book provides our readers (food scientists, nutritionists, biochemists, food technologists, toxicologists, chemists, molecular biologists and public health professionals) with a comprehensive and up-to-date publication on the chemistry, pharmacology and clinical applications of the major herbs, botanicals and teas.

We express our sincere thanks and appreciation to all the contributors who by freely and willingly giving their knowledge and expertise have made this book possible. Our gratitude is also extended to colleagues who have reviewed various chapters and the editorial staff and publishers at Technomic Publishing Company, Inc. for their contribution in bringing this work to publication. Many thanks are also extended to Linda Kerr, Paul Ferguson, Rachel Mazza and Michael Weis who helped with the preparation of portions of the book.

We hope that this book will serve to further stimulate the development of functional foods and nutraceuticals and provide consumers worldwide with products that prevent diseases and help them maintain healthier lives throughout the new millennium.

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CHAPTER 1

Garlic Constituents and Disease Prevention

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1. INTRODUCTION

In recent years, sources of disease-modifying foods and their functional components have attracted attention in nutrition and clinical research. Garlic appears to be a food item that contributes multiple constituents that can potentially benefit human health. Papers, journals and the media have been reporting on various medical findings related to the effects of garlic. Over 2,000 papers have been written about garlic or its constituents (Lawson, 1998a). As a consequence, some health organizations have targeted garlic as a prime candidate for the development of low-cost "functional foods" and "nutraceuticals" that help to reduce risk factors associated with cardiovascular disease and cancer (Block, 1992). Garlic is fast becoming one of the most significant nutraceuticals of our time.

1.1. BACKGROUND INFORMATION

For centuries, garlic has been thought of as a magical healing plant. Its use as a medicinal agent was recorded in Sanskrit documents over 5,000 years ago. Garlic was used by Egyptian slaves during the building of the pyramids and by the Romans who ate it to strengthen themselves during battles. Hippocrates, considered to be the "father of medicine," and Dioscorides, the "father of pharmacy," recommended garlic for several conditions such as infection and blood flow problems. In both world wars, garlic was used to treat wounds when other antibiotics were not available (Fenwick and Hanley, 1985a; Lawson, 1998a). Medicinally, garlic has been used in numerous disorders including

atherosclerosis, hypertension, colds, headaches, worms and tumors (Fenwick and Hanley, 1985a; Murry, 1995).

Botanically, garlic is known as *Allium sativum* L. (family Liliaceae). The exact origin of the genus name is unknown, nevertheless, a relation to the Latin word *olere* is often made, meaning, "to smell." The general characteristics of this genus are plants that are herbaceous perennials and usually form bulbs. Garlic grows to approximately 30–90 cm in height in well-fertilized, sandy, loamy soil in warm sunny locations during spring and summer. Leeks, onions and shallots are all part of the *Allium* genus along with garlic (Murry, 1995; Hahn, 1996).

In 1997, the global production of garlic was 12 million metric tons, with China being the leading producer (8.8 million metric tons), followed by South Korea and India (0.5 million metric tons each). The United States (252,000 metric tons) and Argentina (90,000 metric tons) are the major producers in North and South America, respectively (Food and Agriculture Organization of the United Nations, 1997). The highest producer of garlic per hectare of the above-mentioned countries is the United States (16,816 kg/ha), whereas the largest producer per hectare in the world is Egypt (22,729 kg/ha) with an overall production yield of 159,000 metric tons. In Canada, the commercial production of garlic is a relatively new industry, with Ontario producing 2,220 metric tons during 1998 (87% of the Canadian production) (Ontario Garlic Growers Association, 1998).

In 1994, the U.S. consumption of garlic, primarily as food, was 199 million kg, while in Canada, the 1998 consumption was 10.9 million kg. The U.S. herbal supplement industry is a fast-growing industry with sales doubling from 1994 to 1997. The herbal industry in 1997 had sales of $3.65 billion, of which garlic supplements accounted for $200 million (Monmaney and Roan, 1998). The two major garlic supplement producers are Lichtwer Pharma GmbH, in Germany, whose products are sold under the brand name Kwai, and Wakunaga Pharmaceutical Co. of Japan, whose products are sold under the brand name Kyolic.

Although garlic has been used for centuries in herbal medicine, it is only during the last 15–20 years that some of the health claims associated with garlic (e.g., lowering of blood cholesterol and blood pressure, and its anticancer properties) have been tested rigorously for legitimate scientific merit (Han et al., 1995; Lawson, 1998a). With over 2,000 studies, garlic can be considered one of the most researched medicinal plants. Several comprehensive reviews have been published on garlic (Block, 1985; Fenwick and Hanley, 1985a, 1985b, 1985c; Kendler, 1987; Kleijnen et al., 1989; Block, 1992; Reuter and Sendl, 1994; Agarwal, 1996; Koch and Lawson, 1996; Lawson, 1998a), yet the complex biological and pharmacological actions of garlic and its constituents are still not completely understood. The pace of the biological studies has far exceeded the chemical and analytical studies required for identifying

the constituents and active components in the various garlic preparations used in the *in vitro* and *in vivo* studies (Lawson, 1993).

2. CHEMICAL COMPOSITION AND CHEMISTRY

2.1. COMPOSITION

Garlic is composed mainly of water (56–68%), followed by carbohydrates (26–30%). The most significant components, medicinally, are the organosulfur-containing compounds (11–35 mg/g fresh garlic). Garlic contains nearly three times as much sulfur-containing compounds (per g fresh weight) as broccoli, onions, apricots or cauliflower (Lawson, 1996). The investigation of garlic usually concerns the sulfur-containing compounds, possibly due to their presence in garlic in unusually high amounts or to the pharmacological activities attributed to various sulfur-containing compounds (e.g., penicillin, probucol).

Garlic also contains various other compounds such as saponins, vitamins (ascorbic acid 30 mg/100 g fresh weight, vitamin E 9.4 μg/g), minerals (selenium 0.014 mg/100 g, chromium 0.05 mg/100 g) plus others (Fenwick and Hanley, 1985b; U.S. Department of Agriculture, 1998).

2.2. CHEMISTRY

2.2.1. γ-Glutamyl-Cysteines and Cysteine Sulfoxides

The mature, intact garlic clove contains mainly cysteine sulfoxides such as alliin (5–14 mg/g fresh garlic), followed by methiin and isoalliin that are formed from the γ-glutamyl-cysteines (γ-glutamyl-S-*trans*-1-propenylcysteine, γ-glutamyl-S-allylcysteine and γ-glutamyl-S-methylcysteine) (Figure 1.1). During wintering and sprouting, the γ-glutamyl-cysteines are hydrolyzed to form cysteine sulfoxides by increasing levels of γ-glutamyl-transpeptidase. This process also occurs during storage and occurs more rapidly at cooler temperatures. The cysteine sulfoxides (8–19 mg/g fresh weight) and γ-glutamyl-cysteines (5–16 mg/g) account for approximately 82% of the total sulfur in fresh garlic (Lawson, 1993). When garlic is cut, crushed or chewed, the enzyme alliinase is released, converting the cysteine sulfoxides into the thiosulfinates. The bulb contains most of the cysteine sulfoxides (alliin), approximately 85%, whereas the leaves (12%) and the roots (2%) contain smaller amounts (Lawson, 1998a).

2.2.2. Alliinase

Alliinases or alliin lyases (EC 4.4.1.4) are pyridoxal 5′-phosphate dependent a,b-eliminating lyases that catalyze the conversion of the cysteine sulfoxides

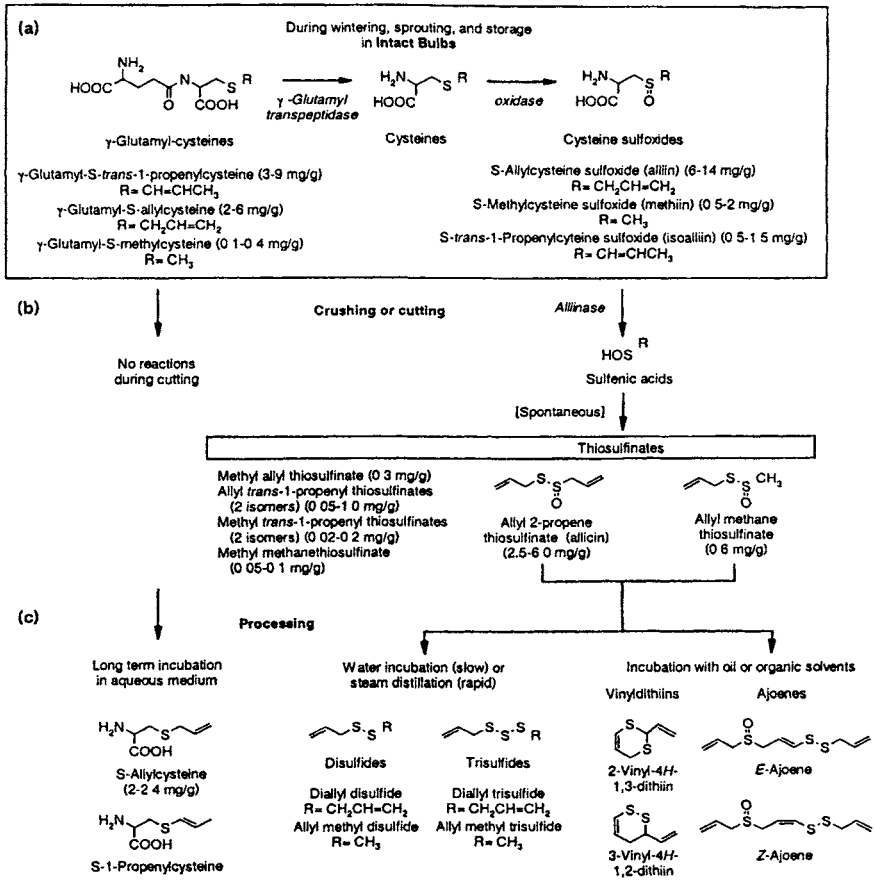

Figure 1.1 Chemical reactions and structures of organosulfur compounds found in (a) intact cloves, (b) upon crushing and (c) through processing [amounts (mg/g) are approximate levels in fresh garlic]. Adapted from Lawson (1996, 1998a).

to the biologically active dialkyl thiosulfinates via sulfenic acid intermediates (Block, 1992). Alliinase is localized to a few vascular bundle sheath cells around the veins or phloem (Ellmore and Feldberg, 1994), whereas alliin and the other cysteine sulfoxides are found in the mesophyll cells. Two different alliinase activities have recently been found in garlic. One is specific for alliin and isoalliin, the other methiin. The former has a pH optimum of 4.5 and cleaves 97% of its substrate within 0.5 min at 23°C, the latter has a pH optimum of 6.5, cleaving 97% of its substrate within 5 min (Block, 1992). Alliinase is temperature and pH dependent and can be irreversibly deactivated at pH 1.5–3.0. This enzyme is approximately 10 times more abundant in the

cloves than in the leaves and accounts for at least 10% of the total protein in the cloves (Ellmore and Feldberg, 1994).

2.2.3. Thiosulfinates

The thiosulfinates (2–9 mg/g fresh crushed garlic) are reactive, volatile, odor producing substances formed enzymatically when garlic is cut, crushed or chewed. Allicin (allyl 2-propenethiosulfinate) is the most abundant thiosulfinate (70%) formed via alliinase reactions, with allyl methanethiosulfinate being the second most abundant (18%). Various other thiosulfinates are formed in low concentrations. Conversion of the cysteine sulfoxides to thiosulfinates via alliinase occurs rapidly, within 0.2–0.5 min at room temperature for allicin and 1.5–5 min for allyl methyl thiosulfinates. The stability of thiosulfinates is dependent on solvent, temperature, concentration and purity. Allicin is soluble in organic solvents, especially polar solvents, but it is less soluble in water. The half-life of pure allicin in water and 1 mM citric acid is 30 and 60 days, respectively. Without a solvent, the half-life of allicin decreases to 16 hours (Lawson, 1993). Thiosulfinates in garlic homogenates are less stable than their pure forms, possibly due to water-soluble substances in the garlic homogenates. Refrigeration greatly increases the stability, but the only long-term storage option is freezing at −70°C (Lawson, 1996).

The thiosulfinates undergo various transformations depending on temperature, pH and solvent conditions, to form more stable compounds such as di- and tri-sulfides, allylsulfides, vinyl dithiins, ajoenes and mercaptocysteines. Incubation of allicin or allyl methane thiosulfinate in low-polarity solvents or without solvents produces mainly vinyl dithiins followed by ajoenes in smaller amounts. Incubation in alcohol gives variable results forming ajoenes, diallyl trisulfide or vinyl dithiins, depending on whether pure allicin or cloves are incubated. Steam distilled garlic oil consists of diallyl disulfide, diallyl trisulfide and mono- to hexasulfides (Lawson, 1993). It should be noted that intact garlic cloves are relatively odorless and do not contain allicin and allicin-generated compounds, although they do contain cysteine sulfoxides and γ-glutamyl-cysteines, precursors of allicin.

2.3. VARIATION IN ORGANOSULFUR COMPOUNDS IN GARLIC

The generation of allicin and other organosulfur compounds varies depending on soil conditions, climate, garlic variety, harvest dates and post-harvest handling. According to Lawson (1998a), the variation among garlic strains typically found in grocery stores (in California) differs 1.8–2.7-fold in alliin and 1.5–4.2-fold in γ-glutamylcysteines. The addition of ammonium sulfate to the soil, as compared to ammonium nitrate, causes a proportional

increase of allicin-releasing potential in garlic bulbs (Reuter and Sendl, 1994; Kosian, 1998).

3. COMMERCIAL GARLIC PREPARATIONS

Several types of garlic preparations are commercially available (see Table 1.1). These products are produced under a variety of conditions such as low-temperature drying, steam distillation, and long-term incubation in various mediums. Most commercial products on the market either generate allicin or contain allicin-derived compounds. Analysis of garlic supplements by HPLC for the estimation of allicin in some of the commercial and noncommercial products are shown in Figure 1.2.

3.1. ALLICIN-DERIVED PRODUCTS

Fresh garlic and powders that have been prepared by careful drying at low temperatures (< 60°C) are capable of producing allicin. These garlic supplements contain the enzyme alliinase and its substrate alliin. The potency of these supplements is usually expressed as "allicin-releasing potential" or "allicin yield," the amount of allicin generated from alliin when the tablet or capsule is exposed to an aqueous medium. The most popular forms of garlic supplements are made from garlic powders and are sold as either enteric-coated tablets (e.g., Kwai) or nonenteric-coated capsules/tablets. Most of the garlic tablets are standardized for their allicin yield. This ensures reproducible dosages of the supplement. The variation in the allicin-releasing potential of the major brands ranges from 0.1 to 8.9 mg/g powder (Han et al., 1995). Drying at higher temperatures often results in irreversible loss of activity of the enzyme alliinase, thereby preventing allicin generation. Garlic powder as a spice may or may not contain active alliinase, depending on drying conditions.

Some products do not generate allicin but contain allicin-derived compounds such as garlic macerates in oil and garlic oil products. These products are often not standardized for organosulfur compounds and contain varying amounts of allicin-derived compounds such as polysulfides, ajoenes and vinyl dithiins.

3.2. NON-ALLICIN-DERIVED PRODUCTS

Although most garlic products rely on allicin-derived compounds for their therapeutic effect, some products do not produce any significant amounts of either allicin or allicin-derived compounds. Aged garlic extracts (e.g., Kyolic) contain γ-glutamylcysteine-derived compounds such as S-allylcysteine (SAC) and small amounts of S-allylmercaptocysteine. Several prod-

TABLE 1.1. **Commercially Available Products and Their Possible Active Principles.**

Products	Processing	Possible Active Principle(s)	Notes
Fresh garlic	None	Allicin and allicin-derived sulfur compounds generated *in vivo*	Heating may cause loss of alliinase activity (>60°C)
Garlic powder tablets (e.g., Kwai, etc.)	Drying/grinding	Allicin and allicin-derived sulfur compounds generated *in vivo*	May be enteric or nonenteric coated and/or standardized for alliin content/allicin-generating potential
Oil-macerated garlic	Incubation in oil	Vinyl dithiins, ajoenes, allyl sulfides	Not often found commercially in North America
Garlic oil	Steam distillation	Allyl di- and trisulfides	
Aged garlic extract (e.g., Kyolic, etc.)	Incubation in ethanol (>1 yr.)	S-allylcysteine (SAC) S-allylmercaptocysteine	Contains no allicin or allicin-derived compounds

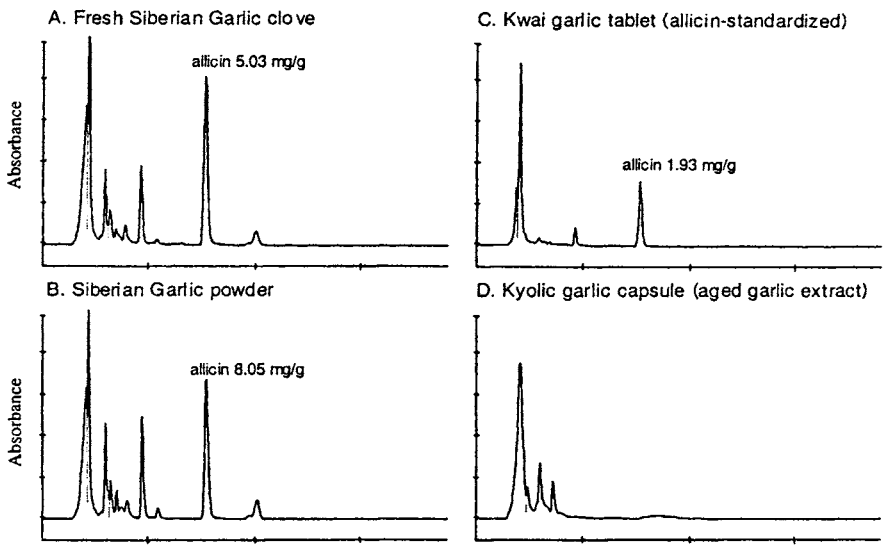

Figure 1.2 HPLC of allicin from (A) Ontario-grown Siberian garlic bulb, (B) dried powder derived from Ontario-grown Siberian garlic bulb, (C) Kwai garlic tablet (Lichtwer Pharma GmbH, Germany) and (D) Kyolic garlic capsule supplement (Wakunaga of America Co , Ltd., USA). Allicin was determined by HPLC using a C-18 reverse-phase column with a 50 50 water·methanol (v/v) eluant, at a flow rate of 1 mL/min (Lawson et al., 1991).

ucts are known to contain a combination of allicin-derived and non-allicin-derived compounds (e.g., Quintessence).

4. METABOLIC FATE OF GARLIC-DERIVED ORGANOSULFUR COMPOUNDS

Many biological studies have shown physiological effects with a variety of garlic preparations, ranging from fresh and powdered garlic to garlic oils, containing various active components. However, it has not been possible to clearly identify the organosulfur metabolites that may be responsible for the physiological effects of garlic. This is primarily due to the fact that the metabolic fate of allicin, generally regarded to be the most important biologically active compound derived from garlic, is not well understood (Lawson and Wang, 1993; Reuter and Sendl, 1994; Freeman and Kodera, 1995; Lawson and Block, 1997; Freeman and Kodera, 1997; Lawson, 1998a).

An abbreviated version of the potential pathways for the metabolism of certain predominant organosulfur compounds derived from garlic is shown in Figure 1.3. This summarizes the events that occur when fresh garlic or commercial garlic preparations are consumed, starting from the chewing/biting and swallowing of garlic, followed by digestion in the stomach and finally moving

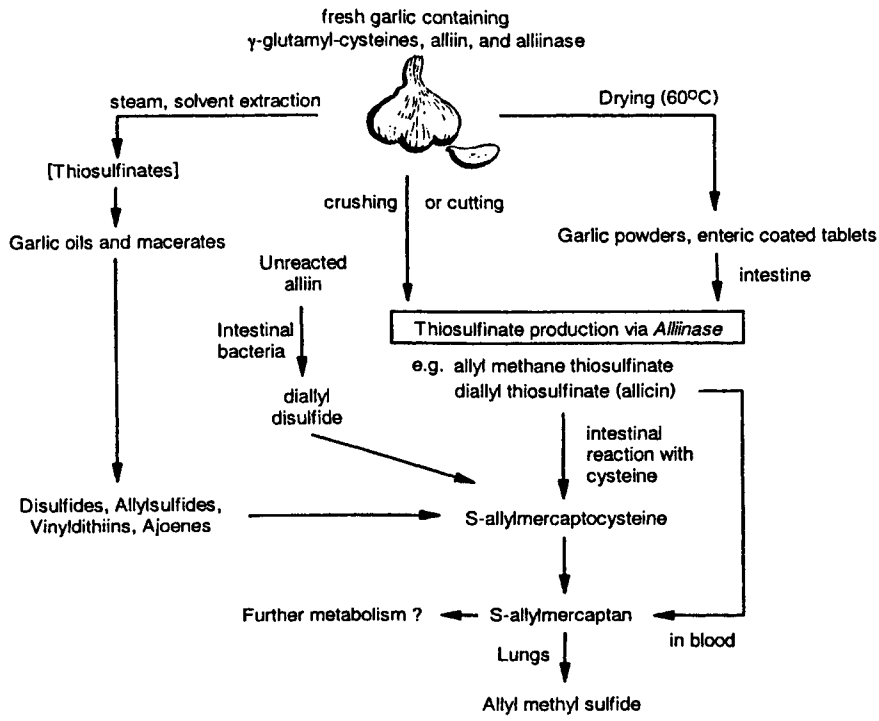

Figure 1.3 Abbreviated scheme for the possible pathways involved in the metabolism of garlic and garlic-derived compounds.

to the intestine. If fresh garlic or garlic powder capsules, which are not enteric-coated, are consumed, alliin would be converted to allicin in the stomach. This would occur only if the acidity of the stomach content is above its normal pH of 1–3, otherwise, the enzyme alliinase would be irreversibly deactivated. Consequently, this often results in inefficient and highly variable generation of allicin required for the physiological effects attributed to garlic. For this reason, it is often recommended that garlic preparations that are enteric-coated be taken. This allows the garlic to withstand the acidic environment of the stomach, and upon reaching the intestine, where the pH is neutral, extensive conversion of alliin to allicin takes place. It has been suggested that alliin that has survived the acidic environment in the stomach could potentially be converted to physiologically-active diallyl disulfide in the intestine (Block, 1992).

At present, the role of allicin in garlic research is controversial (Freeman and Kodera, 1995; Lawson and Block, 1997; Freeman and Kodera, 1997). Although allicin and allicin-derived organosulfur compounds are regarded by most investigators to be responsible for the various physiological effects of garlic, allicin does not remain in the blood for longer than 5 minutes after the

consumption of fresh garlic or commercial garlic preparations (Freeman and Kodera, 1995; Lawson, 1998a). Recent studies have shown that allicin is very unstable in the blood and is metabolized rapidly to form allyl mercaptan, which can potentially be metabolized to further metabolites (Lawson, 1998a).

A recent study has shown the presence of garlic metabolites (e.g., allyl methyl sulfide, dimethyl sulfide and acetone) in the human breath 30 h after garlic consumption (Taucher et al., 1996; Lawson, 1998a). It has been suggested that these metabolites may be associated with the various physiological effects associated with garlic (Lawson, 1998a).

Another potential metabolic pathway for allicin and its transformation compounds is through its reaction with the amino acid cysteine (in the intestine) to form S-allylmercaptocysteine, which is converted to allyl mercaptan. *In vitro* studies have shown physiological activity associated with allyl mercaptan and S-allylmercaptocysteine, which are potential precursors of organosulfur-containing metabolites (Wattenberg et al., 1989; Lee et al., 1994; Gebhardt and Beck, 1996; Pinto et al., 1997; Sigounas et al., 1997a, 1997b). Recent human studies have shown the formation of increased levels of acetone in the breath after consumption of garlic, suggested to be indicative of enhanced metabolism of blood lipids (Taucher et al., 1996).

5. HEALTH BENEFITS

5.1. CARDIOVASCULAR EFFECTS

5.1.1. Cholesterol Lowering

Since 1900, approximately 166 clinical studies have focused on the potential hypocholesterolemic effect of garlic in humans (Reuter and Sendl, 1994; Han et al., 1995; Lawson, 1998a). Most of these studies have used garlic preparations that have either allicin-releasing potential or have allicin-derived organosulfur compounds as their main ingredient (fresh garlic, garlic powders, garlic oils, garlic oil macerates). A combination of a commercial garlic preparation along with an omega-3 fish oil concentrate has recently been reported to offer reductions in cholesterol plus triglyceride levels in moderately hypercholesterolemic subjects (Adler and Holub, 1997). These studies have reported, on average, a lowering of total serum cholesterol by about 10–12%. Two recent meta-analyses of the primary clinical trials have also confirmed the cholesterol lowering effect (~10%) in hypercholesterolemic subjects (Warshafsky et al., 1993; Silagy and Neil, 1994a). A German study found that the cholesterol-lowering effect with garlic powder (900 mg/day) was as effective as the drug bezafibrate (Holzgartner et al., 1992).

The early introduction of garlic supplements with proven clinical efficacy to individuals with normal or slightly elevated cholesterol levels can potentially reduce the progression of their cholesterol values to levels that would require the eventual intervention with beneficial but costly pharmaceutical therapies. However, the new generation of cholesterol-lowering drugs, hepatic HMG-CoA reductase inhibitors (statins), can provide cholesterol reductions of 20–25%. The cost (per 1% reduction of cholesterol) of putting a patient on established pharmaceutical therapies is considerably more than that offered by alternative, clinically proven garlic supplements.

Although it is not yet known which of the compounds in garlic are responsible for the blood cholesterol-lowering effect, there is considerable evidence from animal and other *in vitro* studies to suggest that allicin or allicin-generated organosulfur compounds are responsible for the effect (Gebhardt et al., 1994; Yeh and Yeh, 1994; Gebhardt and Beck, 1996). Tissue culture studies have shown allyl disulfide and allyl mercaptan to inhibit HMG-CoA reductase, the rate-limiting hepatic enzyme for the biosynthesis of cholesterol in the body. The mechanism of this reaction is thought to involve the formation of sulfide bridges within the HMG-CoA reductase enzyme by interaction with the allicin-derived compounds, rendering the enzyme inactive (Gebhardt et al., 1994).

Although there is considerable evidence based on published studies (Kleijnen et al., 1989; Warshafsky et al., 1993; Silagy and Neil, 1994a; Agarwal, 1996; Reuter et al., 1996) in support of the cholesterol-lowering effect of garlic, there is some growing concern about the usefulness of garlic as a cholesterol-lowering supplement. This concern has arisen from recent human clinical studies that have shown no cholesterol-lowering effect (Simons et al., 1995; Berthold et al., 1998; McCrindle et al., 1998; Isaacsohn et al., 1998) and one meta-analysis (Neil et al., 1996) carried out in the UK that found no statistically significant cholesterol lowering due to garlic consumption in human subjects. These studies have created uncertainty about the therapeutic benefits of garlic supplements for lowering cholesterol (Berthold and Sudhop, 1998). It should be pointed out, however, that most of the recent negative results from studies that have used Kwai garlic supplements may be due to a change in the composition of these supplements as suggested by Lawson (1998b).

There are various potential reasons for the lack of effect in the above mentioned studies. One hypothesis may be that (at present) most garlic supplements are sold as dried powder and do not contain preformed allicin, the thiosulfinate that is supposedly required for the cholesterol-lowering effect. Although it is true that the allicin-releasing potential is determined under simulated conditions that are likely to exist in the intestine where the enteric-coated garlic supplements are supposed to disintegrate and generate allicin, conditions in the intestine may vary from one individual to another. Likewise, there may be other factors present in the intestine that may affect the allicin-

releasing potential. This could result in varying amounts of allicin and allicin-derived compounds produced between individuals resulting in varying therapeutic effects, eventually affecting the statistical significance.

Another possibility for lack of effect may be related to dietary factors. Several recent studies that have shown a lack of effect with garlic supplementation used a diet restriction regime (NCEP step I/II diets); in contrast, studies that have shown an effect usually had no dietary restrictions.

5.1.2. Blood Pressure Lowering

The effect of garlic preparations on blood pressure has been studied since 1921. A meta-analysis of controlled human trials using garlic preparations (powders) concluded that garlic significantly (although very modestly) lowers systolic and diastolic blood pressures, although the reductions were generally greater for systolic pressure (Silagy and Neil, 1994b). Although several animal and human studies have demonstrated a moderate hypotensive effect, the precise active principal(s) present in garlic preparations responsible for blood pressure lowering are not known. Sendl and colleagues (1992) have shown that the aqueous extract of garlic leaves, containing mainly glutamyl peptides, exhibits greater inhibitory activity (compared to the bulb) toward the angiotensin-converting enzyme (an important pathway in blood pressure regulation). More recently, it has been shown that aqueous extracts of garlic increase the activity of nitric oxide synthase, an enzyme that produces nitric oxide, which is associated with a lowering of blood pressure (Das et al., 1995a, 1995b; Pedraza-Chaverri et al., 1998). As with aforementioned blood cholesterol trials, not all garlic studies have shown blood pressure reducing effects (Silagy and Neil, 1994b).

5.1.3. Anti-Thrombotic Activity

One of the therapeutic effects of garlic known since 1978 is its potential effect on blood coagulation and platelet aggregation (Lawson et al., 1992; Han et al., 1995), two important factors involved in cardiovascular disease and its associated thrombogenicity. It has been shown through several *in vitro* and *ex vivo* studies (animals and humans) that certain garlic constituents affect platelet aggregation (Makheja and Bailey, 1990; Venton et al., 1991; Lawson et al., 1992; Han et al., 1995). Adenosine, which is present in garlic cloves, is known to inhibit platelet aggregation, but this inhibition was seen only in platelet-rich plasma and was absent in whole blood (Han et al., 1995). Thus, the effect with adenosine may be of questionable physiological relevance (Lawson et al., 1992). Perhaps the most interesting characteristic is the ability of garlic constituents to inhibit the action of most platelet agonists (Venton et al., 1991). A number of garlic preparations (ether extracted garlic oil, steam

distilled garlic oil, fresh garlic, garlic powder, aqueous garlic extract, synthetic and natural allicin, ajoenes) have been used to study the effects of garlic on platelet aggregation (Lawson et al., 1992). The authors have compared the effects of these commercial preparations and identified the important active principles as allicin, ajoenes, vinyl dithiins and diallyl trisulfide. Allicin and diallyl trisulfide were found to be the most active garlic-derived sulfur compounds in inhibiting platelet aggregation *in vitro*. The precise mechanisms for the inhibition of platelet aggregation by garlic-derived compounds are not known but likely include multiple effects such as the inhibition of eicosanoid formation from arachidonic acid (Mohammad and Woodward, 1986; Apitz-Castro et al., 1986, 1991, 1992; Ali et al., 1990; Makheja and Bailey, 1990; Lawson et al., 1992; Srivastava and Tyagi, 1993; Ali and Thomson, 1995; Batirel et al., 1996).

Garlic is also known to have an effect on blood coagulation and fibrinolytic activity (breakup of the fibrin clot into fibrin degradation products). Deficient fibrinolytic activity due to increased plasminogen activator inhibitor type-1 levels or reduced levels of tissue-type plasminogen activator predisposes patients to thrombotic events. Human studies have shown that garlic consumption leads to increased fibrinolytic activity, increased blood coagulation time and decreased fibrinogen levels (Han et al., 1995). A recent study in Germany showed that patients taking a garlic powder supplement had more flexibility in their blood vessels that may provide a cardioprotective effect (Breithaupt-Grogler et al., 1997).

5.2. ANTICANCER PROPERTIES

A role for garlic in the prevention of cancer comes from epidemiological studies that have shown that consumption of *Allium* vegetables such as garlic resulted in a significant reduction in the development of stomach cancer (Lau et al., 1990; Han et al., 1995; Milner, 1996; Lawson, 1998a). An epidemiological study comparing two counties in China determined the incidence of stomach cancer in a region of people consuming 20 g fresh garlic per day was 8% of that where people ate less than 1 g per day (Mei et al., 1982). Members of the stomach-cancer-prone population were shown to have higher levels of nitrites in their gastric juices. Garlic has been shown to have an inhibitory effect on nitroso compound formation *in vivo* by decreasing the amount of nitrate-reducing bacteria in the stomach (Mei et al., 1989). A survey conducted by Hopkins and Williams (1981) found 246 risk factors associated with cardiovascular disease, of which low intake of garlic was a factor. A more recent prospective study in the United States, the "Iowa Women's Health Study" (41,837 women) (Steinmetz et al., 1994), determined an inverse relationship between colon cancer risk and garlic consumption, with an age and energy adjusted relative risk of 0.68.

Studies have indicated that an average intake of 4–6 g of garlic per day is inversely correlated with the incidence of gastrointestinal cancers (Lawson, 1998a). Although several epidemiological studies have proposed an anticancer role for garlic, three epidemiological studies from the Netherlands have found no association between garlic supplement consumption and the prevention of breast, colon and lung cancers (Dorant et al., 1994, 1995, 1996).

Numerous *in vitro* and *in vivo* studies have determined that various constituents of garlic inhibit tumor growth and possess other anticancer properties (Lau et al., 1990; Brady et al., 1991; Han et al., 1995; Milner, 1996; Schaffer et al., 1996; Srivastava et al., 1997; Pinto et al., 1997; Riggs et al., 1997; Hu and Singh, 1997; Sakamoto et al., 1997; Lawson, 1998a). The allyl-containing organosulfides such as diallyl di- and trisulfides, allyl methyl di- and trisulfides, allylmercaptan, and S-allylcysteine as well as fresh garlic extract have inhibitory effects on the formation of tumors induced by various initiators (Dorant et al., 1993; Heber, 1997). Mice treated with thiosulfinates or freshly ground garlic showed no tumor formation after being injected with ascites tumor cells. Furthermore, injections directly into existing tumors stopped their growth (Fujiwara and Natata, 1967). Studies with aged garlic extract fed to rats at 2–4% of the diet decreased carcinogen-induced tumors and DNA adducts. The active principle is unknown, but it has been postulated that S-allylcysteine may be the effective compound (Liu and Milner, 1990). Garlic-derived organosulfides have also been shown to inhibit the conjugation of carcinogens with mammary cell DNA to form DNA adducts. Changes in the DNA adducts serve as an early indicator of the alterations in the initiation phase of carcinogenesis. The anticarcinogenic action of these organosulfides may be caused by a direct inhibition of tumor cell metabolism (cytochrome P-450), inhibition of initiation/promotion through carcinogen detoxification via increased glutathione S-transferase or through modulation of host immune response (Lau et al., 1990; Brady et al., 1991; Srivastava et al., 1997; Pinto et al., 1997).

5.3. ANTIOXIDANT EFFECTS

Garlic has antioxidant effects. Aged garlic extract has been shown to inhibit lipid peroxidation in rat liver microsomes in a dose-dependent manner (Horie et al., 1992). S-allylcysteine and S-allylmercaptocysteine also have radical scavenging activities (Imai et al., 1994). One human clinical trial with garlic supplementation (Kwai, 600 mg/d) resulted in less susceptibility to copper-induced oxidation in low-density lipoprotein (Phelps and Harris, 1993). Allyl mercaptan generated by blood cells also appears to have an antioxidant effect *in vivo* (Lawson and Wang, 1993). The antioxidant effect is useful in cancer prevention as well as in the prevention of cardiovascular disease. Garlic also prevents the oxidation of low-density lipoprotein (LDL) to the atherogenic oxidized LDL species in human subjects (Phelps and Harris, 1993).

5.4. ANTIMICROBIAL EFFECTS

In addition to cardiovascular and anticancer effects, which are well documented in peer-reviewed journals, there are other known physiological effects of garlic. The antibacterial and antifungal activities (against a variety of Gram-negative and Gram-positive microorganisms) of garlic have been attributed to allicin. The antibiotic activity of 1 mg of allicin has been equated to that of 15 IU of penicillin (Han et al., 1995). Ajoene, an organosulfur compound derived from garlic, has been shown to increase the antimalarial activity of chloroquine against the parasite *P. berghei* (Perez et al., 1994). Recent studies have also demonstrated an inhibitory effect by aqueous garlic extracts on *Helicobacter pylori*, a bacterium implicated in the etiology of stomach cancer (Cellini et al., 1996; Sivam et al., 1997).

6. ADVERSE EFFECTS

Garlic is widely used as a food and is not considered dangerous, in fact it is "Generally Regarded as Safe" (GRAS) by the FDA. However, various negative effects can occur after garlic consumption that are generally nonlife-threatening but are discomforting and embarrassing for some individuals. Odoriferous garlic breath and perspiration often occur after garlic consumption due mainly to the allyl sulfide compounds (allyl methyl sulfide, disulfide, diallyl sulfide, disulfide and 2-propenethiol, which are expelled from the blood via the lungs or by sweat) (Block, 1992). Garlic may also cause an acid reflux effect that is due to transient lower-esophageal sphincter relaxation (Koch, 1996). A few individuals may experience allergic-type reactions (e.g., dermatitis, asthma) to fresh garlic or diallyl disulfide (Asero et al., 1998; Jappe et al., 1999).

On the other hand, a controversy exists about possible adverse effects of allicin According to some researchers, synthetic allicin (0.2 mg/mL), when tested *in vitro*, is considered toxic based on their observations of the oxidation of blood hemoglobin (Freeman and Kodera, 1995). The LD_{50} of allicin in mice is 60 mg/kg intravenously and 120 mg/kg subcutaneously (Block, 1992). Aqueous extracts of allicin have also been shown to have adverse effects in mice at 300–600 mg/kg/d (Fehri et al., 1991). These dosages are several hundredfold above any normal dose that would generally be ingested by humans. Moreover, garlic only produces on average 2–6 mg allicin/g, which at normal garlic consumption is well below the toxic dose.

In general, based on numerous human clinical trials and studies, fresh garlic ingestion at 10 g per day (one to two cloves or 4–6 g garlic powder) is considered safe when taken with a meal (Koch, 1996). For all intents and purposes, garlic is considered safe and nontoxic.

7. CONCLUSION

Garlic and derived preparations are often touted as having (or not having) certain physiological effects based on research and clinical studies. Not all studies have employed the same preparations of garlic; rather, products such as powders, oils, macerates and aged garlic extracts have often been used, all containing varying levels of potential physiologically active compounds. Yet, the physiological effects of these garlic products are often grouped together as the effects of garlic and are not separated depending on the type of preparation used. Consequentially, the physiological effects from "garlic" are highly unpredictable and inconsistent. Therefore, ideally, all studies need to be examined in relation to their active components and/or to the specific garlic preparation used and should not be pooled together as the effect of garlic in general.

It is becoming apparent that the physiological/clinical effects (e.g., blood cholesterol lowering) of the same or different garlic preparations can vary considerably across different groups of subjects. These apparent inconsistencies may be due to several factors including dose and duration, dietary components (e.g., fat levels), variability in the levels of the different organosulfur compounds in the garlic supplements, and variability between individuals to generate, *in vivo*, the physiologically active metabolites from the garlic supplements. More research is needed to address these inconsistencies and to demonstrate that the physiological effects are reliable and reproducible for a given garlic preparation. Consumers using garlic preparations for lowering blood cholesterol should be encouraged to have their serum lipids monitored to ensure clinical efficacy of the supplement. Support for the anticancer effects of garlic must await results of further *in vitro* studies and, particularly, human clinical trials. The aforementioned inconsistencies in study results need to be dealt with by developing more reliable and defined garlic products (supplements and functional foods) with dependable and reproducible clinical efficacy.

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5 CHEMISTRY AND PHARMACOLOGY OF FENUGREEK

galactomannan are used as thickeners, texture improvers or emulsifiers in the

food-processing industry, especially in low-fat products. In terms of functions for the plant, it has been shown that galactomannan

is hydrolyzed during germination, and the free simple sugars can then be

metabolized and even temporarily transformed into starch in the cotyledons.

In addition to its water retention properties, galactomannan can act as a

water reservoir, buffering the germinating embryo against desiccation during

subsequent temporary periods of drought (Reid and Bewley, 1979).

2.7. OTHER CONSTITUENTS In a recent study on isoflavonoid and lignan content of fenugreek, Mazur

et al. (1998) found that the levels of phytoestrogen were very low and ranged

from 8.6 |xg/100 g for secoisolariciresinol to 9.8 mg/100 for genistein and

10.2 mg/100 g for diadzein, respectively. Fenugreek has also been suggested as a potential source of natural antioxi

dants (Hettiarachchy et al., 1996).

3. PHARMACOLOGY OF FENUGREEK

3.1. EFFECTS ON GLUCOSE HOMEOSTASIS Fenugreek seeds are traditionally known to have antidiabetic properties

(Moissides, 1939). The glucose lowering effect was demonstrated in vivo and

attributed to the defatted fraction of the seed, which

induced hyperglycemia

in normal animals and decreased hyperglycemia and glycosuria in alloxan

induced diabetic dogs (Ribes et al., 1984). These properties were further

investigated in insulin-dependent diabetic dogs, and the testa and endosperm

fraction of the seed, which has high viscosity and is particularly rich in fibers,

was found to be the active subfraction (Ribes et al., 1986). A dose-related

hypoglycemic effect was also noted in normal and alloxan-induced diabetic

rats (Khosla et al., 1995) as well as in normal and alloxan-diabetic mice

(Ajabnoor and Tilmisany, 1988). Clinical studies with fenugreek seed revealed an improvement of glucose

tolerance in healthy volunteers (Sharma, 1986) as well as in type 2 (non-insulin

dependent) diabetic patients (Madar et al., 1988; Sharma and Raghuram,

1990). In 60 type 2 diabetic patients, a diet containing fenugreek seed powder

administered for 24 weeks lowered fasting blood glucose levels, glycosylated

hemoglobin and urinary sugar excretion and improved glucose tolerance at

lower plasma insulin levels (Sharma et al., 1996b). In a recent placebo

controlled study, fenugreek did not affect fasting or postprandial blood sugar

in healthy individuals; it significantly reduced glycemia in mild but not in

severe cases of type 2 diabetes (Bordia et al., 1997). Moreover, in type 1

(insulin-dependent) diabetic patients, a fenugreek diet reduced fasting blood

sugar and urinary glucose excretion and improved glucose tolerance (Sharma

et al., 1990). The antidiabetic effect of fenugreek has been attributed mainly to the high

fiber content of the seeds, with slower gastric emptying and subsequent reduc

tion of glucose intestinal absorption (Madar and Thorne, 1987). The soluble

dietary fiber fraction in fenugreek, with galactomannan as a major constituent,

was shown to reduce glycemia after glucose ingestion (Ali et al., 1995). Hence,

by slowing gastric emptying and forming a nonabsorbable viscous gum when

mixed with water (viscosity effect), the fiber may reduce or delay intestinal

absorption of glucose, which subsequently may improve glycémie control. The major alkaloid trigonelline was previously reported to have a hypoglyce

mic effect (Mishkinsky et al., 1967; Shani et al., 1974). Fenugreek was also

reported to increase insulin binding sites of erythrocytes, and it may improve

peripheral glucose utilization (Raghuram et al., 1994). In most studies with animals or human subjects, a significant reduction in

plasma glucose concentrations following fenugreek administration has been

observed with no significant elevation or even with a reduction in plasma

insulin concentrations. However, after subchronic administration of a fenu

greek seed extract in normal rats, an increase in morning plasma insulin level

was observed in overnight fed animals, suggesting the presence of an insulin

stimulating compound in the extract (Petit et al., 1993). Indeed, the amino acid

4-hydroxyisoleucine was extracted and purified by sequential chromatography

from defatted fenugreek seeds. This compound is not found in mammalian

tissues and is only present in plants, particularly in Trigonella species. It

induced concentration-dependent stimulation of insulin secretion in vitro from

rat incubated Langerhans islets in the micromolar range of concentrations

(200-1,000 fxmol/L). At 200 u.mol/L concentration, 4-hydroxyisoleucine in

duced a biphasic insulin response in rat isolated perfused pancreas in the

presence of a slightly stimulating glucose concentration (8.3 mmol/L) (Sau

vaire and Ribes, 1992-1994). It was ineffective in the presence of 5.0 mmol/

L glucose and induced a glucose-dependent response in the presence of inter

mediate to high glucose concentrations (Sauvaire et al., 1998) (Figure 5.3).

The amino acid was effective in vivo in conscious fasted dogs in improving

oral glucose tolerance after oral administration (Hillaire-Buys et al., 1993)

and in type 2 diabetic rats (Broca et al., 1998). It also increased insulin

secretion in human islets at micromolar concentrations, similar to results

obtained in animal models (Fernandez-Alvarez et al., 1996; Sauvaire et al.,

1998). The coupling mechanism of the secretagogue action of 4-hydroxyisoleu

cine remains to be clearly established. The drug only partially affects the

diazoxide-induced increase in potassium permeability of the B cell plasma

membrane in the presence of 3.0 mmol/L glucose, without any significant

Figure 5.3 Kinetics of insulin secretion of 4-hydroxyisoleucine (200 mmol/L) in the presence

of different glucose concentrations (5, 6.6, 8.3 and 10 mmol/L) Adapted from Sauvaire et

al. (1998). 121

effect per se (Petit et al., 1995b). However, it potentiates the effect of intermedi

ary glucose concentrations on calcium signaling (Petit et al., 1997). Concerning

structure-activity relationships, it was reported that the major natural isomer

of 4-hydroxyisoleucine (2S, 3R, 4S), representing 97% of the compound in

seeds, is the most potent structural analogue tested so far (Sauvaire and Ribes,

1996-1997). However, different synthetic monomethylated derivatives have

been found to be more potent than leucine and isoleucine (Ribes et al., 1996). Most studies on fenugreek have

focused on the effects of the seeds. In

subjects fed a diet of fenugreek leaves, there were no significant differences

in either blood glucose or insulin levels as compared to subjects fed a control

diet (Sharma, 1986). In contrast, a recent investigation reported a hypoglycemic

effect of an aqueous extract of fenugreek leaves when given orally or intraperi

toneally in normal and alloxan-induced diabetic rats (Abdel-Barry et al., 1997). Taken together, these data highlight the potentially beneficial effect of

fenugreek seed as a nutritional supplement in the management of diabetes

and of some of its components for pharmaceutical uses.

3.1.1. Effects on Plasma Cholesterol Concentration
Fenugreek was shown to have hypocholesterolemic properties (Singhal et

al., 1982) and was shown to prevent a diet-induced cholesterol elevation in

rats (Sharma, 1984). In normal and diabetic dogs, a lipid extract was ineffective,

and the hypocholesterolemic effect was attributed to the defatted part of the

seeds, which is rich in fibers and contains steroid saponins (Valette et al., 1984).

The fiber-rich fraction (testa and endosperm) induced an hypocholesterolemic

effect, and the saponin- and protein-rich fraction (cotyledon and axes) was

shown to reduce plasma cholesterol and triglyceride levels (Ribes et al., 1987).

Saponins, alone or with diosgenin, were further shown to be implicated in

the hypocholesterolemic effect of fenugreek seeds (Sauvaire et al., 1991).

Saponins have also been identified as the hypocholesterolemic component of

fenugreek seeds, interacting with bile salts in the digestive tract (Stark and

Madar, 1993). On the other hand, polysaccharides derived from fenugreek

were reported to decrease the uptake of bile acid, i.e., the reduced efficiency

of enterohepatic circulation increases bile acid excretion and may lead to

decreased plasma cholesterol levels (Madar and Shomer, 1990). Gaiactoman

nans from fenugreek have been shown to lower cholesterol concentrations in

liver and blood plasma, and decrease the rate of hepatic synthesis of cholesterol

(Evans et al., 1992). In a clinical study in hyperlipidemic nondiabetic subjects, incorporation of

defatted fenugreek in the diet resulted in a significant reduction of serum total

cholesterol, LDL- and VLDL-cholesterol as well as triglyceride levels. HDL

cholesterol levels were not altered, but the ratio with total cholesterol and

LDL and VLDL cholesterol were significantly increased, suggesting a benefi

cial effect in the lipid profile (Sharma et al., 1991). Fenugreek seeds have Pharmacology of Fenugreek

been foun d to exert hypocholesterolemic activity in diabetic patients (Sharma

et al., 1990). In type 2 diabetic patients, administration

of fenugreek seed

powder resulted in a sustained and long-lasting reduction of total cholesterol,

LDL- and VLDL-cholesterol and triglyceride levels, while a slight but insignif

icant rise in HDL-cholesterol was observed (Sharma et al., 1996c). These data suggest that some fenugreek components may have a beneficial

effect on the lipid profile of diabetic subjects. The ability of fenugreek to

reduce the LDL and VLDL fractions of total cholesterol may be beneficial

in preventing atherosclerosis.

3.2. OTHER PHARMACOLOGICAL EFFECTS Fenugreek seeds are traditionally assumed to have restorative and nutritive

properties. When used as a dietary supplement, Fenugreek seeds did not alter

the food intake of animals (Udayasekhara Rao et al., 1996). In a long-term

study in type 2 diabetic patients, food consumption and mean energy intake

during control and experimental (fenugreek seed administration) periods were

reported to be almost similar and constant with no significant change in

body weight (Sharma et al., 1996a). However, continued administration of a

fenugreek seed extract in rats was shown to increase the animal's appetite

and food consumption (Petit et al., 1993). A regular treatment with purified

steroid saponins from fenugreek seeds was shown to increase food intake and

appetite in normal rats, while modifying the circadian rhythm of feeding

behavior. This treatment was able to stabilize food consumption in diabetic

animals, resulting in progressive weight gain in these animals, as compared

to untreated diabetic controls (Sauvaire et al., 1994; Petit et al., 1995a). An aqueous extract of fenugreek leaves was recently shown to produce

antinociceptive effects in a dose-dependent manner in rats, and both central

and peripheral mechanisms were suggested (Javan et al., 1997).

3.3. ADVERSE EFFECTS AND TOXICITY Among the various antidiabetic plants that have been proposed, fenugreek

is generally considered to be nontoxic (Maries and Farnsworth, 1995). A

safety evaluation of fenugreek seeds was performed in rats whose diet was

supplemented with fenugreek seed flour (up to 20%) for 90 days; fenugreek

appeared to be essentially nontoxic (Udayasekhara Rao et al., 1996). In a 24

week clinical investigation in type 2 diabetic patients, a diet containing 25 g

fenugreek seed powder appeared to be suitable, i.e., some patients complained

initially of minor gastrointestinal symptoms, such as diarrhea and excess

flatulence, which subsided after a few days, and no other adverse effect was

observed (Sharma et al., 1996a, 1996b, 1996c). TABLE 5.6. Principal Pharmacological Effects of Fenugreek.

1 Reported Activity

1 Glucose homeostasis (antidiabetic and/or insulinotropic properties) Lipidemia (hypocholesterolemic and hypotriglyceridemic properties) Feeding behavior (food intake and motivation to eat) Nociception (antinociceptive properties) Species Dogs Rats Mice Humans Rats Dogs Humans Rats Rats Reference Ribeset a l , 1984 Ribes et al , 1986 Shani et al , 1974 Khosla etal., 1995 Abdel-Barry e t a l , 1997 Sauvaire et a l , 1998 Broca et al., 1998 Ajabnoor and Tilmisany, Sharma, 1986 Madar et a l , 1988 Sharma and Raghuram, Fernandez-Alvarez et al Sharma et a l , 1996b Bordia e t a l , 1997 1988 1990 1996 Singhal etal , 1982 Sharma, 1984 Madar and Shomer, 1990 I Evans et a l , 1992 Stark and Madar, 1993 Valette e t a l , 1984 Ribes etal., 1987 Sauvaire et a l , 1991 Sharma and Raghuram, Sharma etal., 1990 Sharma e t a l , 1991 Sharma etal., 1996c Petit etal , 1993 Petit et a l , 1995a Javan e t a l , 1997 1990

124 Allergic reactions after consumption of spices are well known. Two cases

of allergy to fenugreek after inhalation of the seed powder or after skin

application of a paste were recently documented (Patil et al., 1997).

4. CONCLUSIONS Fenugreek seeds contain several compounds such as 4-hydroxyisoleucine,

trigonelline as well as certain aromatic compounds and steroidal substances

that have not been found in other plants. All of these components, alone or

in combination, provide this plant with a number of pharmacological and

therapeutic properties (Table 5.6). In addition, fenugreek is one of the few

plants of the Leguminosae family that contains diosgenin, a key component

used for producing steroidal drugs through hemisynthesis. It is, thus, quite

likely that the components of fenugreek will find applications for the treatment

and prevention of a wide range of diseases.

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6 CHEMISTRY, PHARMACOLOGY AND CLINICAL APPLICATIONS OF ST. JOHN'S WORT AND GINKGO BILOBA

1 3 5

Figure 6.2 Structure of hypericin.

quercetin (2%); the flavonol glycosides hyperoside, also called hyperin (0.7

1.1 %); quercitrin (0.3-0.5%); isoquercitrin (0.3%); amentoflavone, also known

as 13', II8-biapigenin (0.01-0.05% in flowers); I3-II8-biapigenin (0.1-0.5%);

and luteolin and rutin (0.3%) (Razinskaite, 1971; Hobbs, 1989; Nahrstedt

and Butterweck, 1997; Cracchiolo, 1998). Comparative HPLC analyses of

amentoflavone and hypericin in extracts of H. perforatum, H. hirsutum, H.

patulum and H. olympicum have been reported by Baureithel et al. (1997).

Flavonoids are potent antioxidants, free radical scavengers and metal chelators,

and they inhibit lipid peroxidation. Certain biflavonoids including amentofla

vone (Figure 6.3) were previously reported to have inhibitory effects on the

group II phospholipase A2 activity. Amentoflavone has also been found to

inhibit cyclooxygenase from guinea pig epidermis without affecting lipoxygen

ase, and it showed potent anti-inflammatory activity in the rat carrageenan

paw edema model (Kim et al., 1998). Epidemiological studies show an inverse

correlation between flavonoids and mortality from coronary heart diseases

(Cook and Samman, 1996). St. John's Wort

Figure 6.3 Structure of amentoflavone, bilobetin, ginkgetin, isoginkgétin and sciadopitysin.

1.2.4. Essential Oils

Essential oils constitute about 0.06-0.35% of the plant. The major compo

nents are 2-methyloctane (16.4%), a-pinene (10.6%), 0-pinene, limonene,

myrcene, caryophyllene and humulene. Trace components include 2-methylde

cane, 2-methybutenol, undecane and various n-alkanes and n-alkanols (Mathis

and Ourisson, 1964; Sticher, 1977; Chialva et al. 1981; Upton, 1997). A

number of these monoterpenes have antitumoral activity. For example, d

limonene has been shown to have chemopreventive activity against rodent

mammary, skin, liver, lung and forestomach cancer (Crowell, 1999).

1.2.5. Other Constituents

Proanthocyanidins or condensed tannins are found in large concentrations

in the aerial portions of the plant and consist primarily of dimers, trimers,

tetramers and high polymers of catechin and epicatechin. These chemicals are

known to posses antioxidant, antimicrobial, antiviral and cardioprotective

effects (Cook and Samman, 1996; Hollman et al., 1996).

Other bioactives

known to occur in St. John's wort are phytosterols, the coumarins umbellifone

and scopoletin, xanthones, carotenoids and several phenolic acids including

caffeic, chlorogenic, p-coumaric, ferulic, isoferulic and gentisic acid (Mathis

and Ourisson, 1964; Costes and Chantai, 1967; Hobbs, 1989; Upton, 1997).

Xanthones are found primarily in the roots and have been shown to inhibit

MAO-A and MAO-B enzymes (Suzuki et al., 1984).

1.3. COMMERCIAL PREPARATIONS

A variety of St. John's wort preparations are commercially available (Table

6.2). These products are produced under a variety of conditions such as air

drying, olive and/or sunflower oil extraction and extraction with other media

including water, ethanol, methanol, glycerol and supercritical carbon dioxide.

Tea bags containing two grams of the raw herb are also available. A St. John's

wort tea is normally made by pouring about one cup of boiling water over

two teaspoons (2-4 grams) of chopped raw herb, steeping for 5-10 minutes,

then straining.

Little is known about the impact of processing and storage stability on the

quality of St. John's wort products. The reason is the uncertainty of the

various components of St. John's wort that may be responsible for the desired

biological effects, especially antidepressant effects. Until recently, it was be

lieved that the antidepressant activity was related to the level of hypericin

(Suzuki et al., 1984). Now, it appears that the antidepressant constituent of

Hypericum is hyperforin (Chatterjee et al., 1998a, 1998b; Dimpfel et al.,

1998; Laakmann et al., 1998; Muller et al., 1998). Nonetheless, most of the

commercially available St. John's wort products are still standardized to a

certain content of hypericin (Table 6.2), and the few studies on the influence

of processing and storage stability on the quality of St. John's wort products

have used hypericin as the marker compound. Thus, Adamski and Styp

Rekowska (1971) reported that the hypericin content of juice of//, perforatum

and powdered extract dropped by 14% during one year, and the dry extract

remained stable, when stored at 20°C. When stored at 60°C, the hypericin

level decreased by 33%, 33% and 47% from powder extract, tablets and liquid

juice, respectively. Similarly, Araya and Ford (1981) reported that drying of

H. perforatum plants in sunlight destroyed 80% of hypericin.

Similarly, very little is known about the influence of different cultivation

methods on the biochemical activities of Hypericum extracts. According to

Denke et al. (1999), nitrogen fertilization and cultivar influence yield and

quality of the plant material. In this experiment, the most active extract was

from plants of a broad-leaf cultivar that were non-fertilized with nitrogen and

that were extracted with methanol. In another recently published study, Büter

et al. (1998) reports that genetic factors strongly affect plant yield and concen

tration of secondary metabolites of Hypericum. Thus, HPLC analysis of ament

oflavone, biapigenin, hyperforin, hypericin, hyperoside, pseudohypericin,

quercetin and rutin contents were significantly lower in the first year of TABLE 6.2. Commercially Available St. John's Wort Products. Product Fresh herb Dried herb Juice Oil-extracted Water-extracted Ethanol-extracted Ethanol-water-extracted Methanol-extracted Carbon dioxide-extracted Tablets Capsules (NW) Capsules (NS) Capsules (CSN) Liquid concentrate Processing None Drying Extraction Grinding/extraction Grinding/extraction Grinding/extraction Grinding/extraction Grinding/extraction Grinding/extraction Drying/grinding Grinding/extraction Grinding/extraction Grinding/extraction Grinding/extraction/concentration Content of St. John's Wort and Notes na 1 '"~ — — — — — — — — — 375 mg/*/cap; 2-3 caps/days; $0.16/cap; recommended for modulation of mood 400 mg/cap; 3 caps/day; $0.25/cap 300 mg/cap; 3 caps/day; $0.15/cap Ethanol-water-extract: $21.50/2 oz. bottle ra Not available. *375 mg St. John's wort leaf and stem extract standardized to 0.3% hypericin delivering 0.9 mg hypericin. According to several published reports, the standard dose is 2 to 4 grams of raw herb or 0.2 to 1.0 mg of extracted hypericin/day; prices are in US $ for selected products sold on the Internet in August 1999.

cultivation ranging from 12% (hyperin) to 83% (hyperforin) of the contents

measured in the two-year-old crop.

Almost all clinical studies have been conducted using a methanol extract

manufactured by Willmar Schwabe GmbH & Co. of Karlsruhe, Germany, and

marketed under the trade name of Jarsin (TM). This is also called LI 160 and

research grade Hypericum. It should be noted that the alcohol used in the

extraction process is removed from the end product that is essentially methanol

free. At the present time, it is not known if other products, such as chopped

raw St. John's wort or a tea, are effective in treating depression or other

illness.

1.4. PHARMACOLOGICAL EFFECTS

1.4.1. Antidepressant Action

The recent increase in popularity of St. John's wort in North America is

due primarily to its antidepressant action. This increase in popular attention

came as a result of a June 1997 positive feature on the herb by ABC television

news magazine program 20/20. Nevertheless, St. John's wort does indeed

appear to be effective in the treatment of mild to moderate cases of depression

(Linde et al., 1996; Hippius, 1998).

Some of the most convincing evidence for the efficacy and safety of St.

John's wort was published in the British Medical Journal by Linde et al.

(1996). The aim of this study was to investigate if extracts of H. perforatum

are more effective than placebo in the treatment of depression, are as effective

as standard antidepressive treatment and have fewer side effects than standard

antidepressant drugs. In the study, the authors conducted a systematic review

and meta-analysis of 23 randomized trials including a total of 1,757 outpatients

with mainly mild or moderately severe depressive disorders: 15 (14 testing

single preparations and one a combination with other plant extracts) were

placebo controlled and eight (six testing single preparations and two combina

tions) compared Hypericum with another drug treatment. The results revealed

that Hypericum extracts were significantly superior to placebo (95% confi

dence interval) and similarly effective as standard antidepressants. There were

two (0.8%) dropouts for side effects with Hypericum and seven (3.0%) with

standard antidepressant drugs. Side effects occurred in 50 (19.8%) patients

on Hypericum and 84 (52.8%) patients on standard antidepressants. It was

concluded that extracts of Hypericum are more effective than placebo for the

treatment of mild to moderately severe depressive disorders.

The herbal preparations used in the studies reviewed by Linde et al. (1996)

were standardized on the basis of hypericin content, and most used the metha

nol extract of Hypericum called LI 160 or Jarsin. However, in the reviewed

studies, the dose of the whole herb varied considerably (from 300 mg to 1,000

mg/day) as did the dose of hypericin (0.4 to 2.7 mg/day), and all of the studies St. John*s Wort

were of short duration. Most were four to eight weeks in length. Also, most

antidepressants become effective after a few weeks; but it may take longer

than eight weeks for antidepressants to build up to maximum effectiveness

in certain individuals. Similarly, the doses of antidepressants used in the

control groups were relatively low, and it is not clear as to how effective St.

John's wort is when compared to a high dose of the synthetic drugs. In

addition, even though the products tested were standardized for hypericin

content, it now appears that hypericin is not among the phytochemicals respon

sible for St. John's wort's antidepressant effects.

In a separate commentary accompanying the Linde et al. (1996) article, De

Smet and Nolen (1996) called for further studies aimed at finding the most

effective treatment dose of Hypericum and for longer studies to evaluate the

risk of relapse and late-emerging side effects. They also called for trials in

severely depressed patients.

In 1997, the National Institutes of Health (NIH) launched the first U.S.

clinical trial of St. John's wort. The three-year study, sponsored by NIH's

Office of Alternad ve Medicine (O AM), the National Institute of Mental Health

(NIMH) and the Office of Dietary Supplements (ODS), will include 336

patients with major depression who will be randomly assigned to one of three

treatment arms for an eight-week trial. One-third of the patients will receive

a uniform dose of St. John's wort, another third will be given placebo and

the final third will take sertraline (Zoloft), a selective serotonin reuptake

inhibitor (SSRI). This study will permit the use of relatively high doses of

both St. John's wort and sertraline and will include severely depressed patients

(NIH, 1997). This study will use a standardized preparation containing a 900

mg daily dose of the herb. In addition, study participants who respond posi

tively will be followed for another 18 weeks. The goal of the follow-up is to

determine if patients given St. John's wort have fewer relapses than patients

given placebo.

Very recently, the findings of a randomized double-blind multicenter com

parative study were reported by Lenoir et al. (1999). The aim of this study

was to investigate the efficacy and tolerability of a new standardized fresh

plant extract obtained from the shoot tips of St. John's wort in the treatment

of mild to moderate depression. Outpatients (348 total: 259 female, 89 male)

with mild to moderate depression were given one tablet of a Hypericum

preparation standardized to either 0.17 mg (114 patients), 0.33 mg (115 pa

tients) or 1 mg (119 patients) total hypericin per day for six weeks, three

times a day. The main outcome measure was the Hamilton Psychiatric Rating

Scale for Depression, which at the end of treatment decreased from 16-17 to

8-9. That is, a relative reduction of about 50% was observed in all groups (280

patients, for protocol analysis). Overall, the intergroup comparison revealed no

significant differences, indicating that this Hypericum preparation was effec

tive at all three doses. Tolerability was found to be excellent, with mild adverse

reactions occurring in only seven of the 348 patients (2%).

The finding by Lenoir et al. (1999) that their Hypericum preparation was

equally effective at the 0.17, 0.33 and 1.0 mg total

hypericin dose per day

clearly suggests that antidepressant effects do not appear to be directly related

to the intake of hypericin. Hyperforin, on the other hand, has recently been

shown to be a dose-related marker for antidepressant efficacy in humans

(Dimpfel et al., 1998; Shellenberg et al., 1998; Laakmann et al., 1998).

Shellenberg et al. (1998) conducted a randomized, double-blind and placebo

controlled parallel-group study on three groups of 18 volunteers each to

determine the effects on EEG of two extracts with the same hypericin content

and differing amounts of hyperforin. They found no effects on EEG for

hypericin, but they found the effects on EEG to be proportional to hyperforin

content. They suggest that Hypericum products containing high amounts of

hyperforin have a "shielding" effect on the central nervous system. The same

team observed a similar effect in rats (Dimpfel et al., 1998).

Results from a randomized, double-blind and placebo-controlled multicenter

study (on the clinical efficacy of extracts of St. John's wort in 147 outpatients

suffering from mild or moderate depression) reported by Laakmann et al.

(1998) also showed that patients receiving a 5% hyperforin solution exhibited

the largest reduction in depression followed by the group receiving 0.5%

hyperforin and then by the placebo group.

Thus, the likely constituent of Hypericum responsible for the antidepressant

effects is hyperforin, a unique phloroglucinol derivative found in the plant

(Chatterjee et al., 1998a, 1998b). The mechanism by which St. John's wort

exerts antidepressan t effects is unclear. However, according to recent reports

(Muller et al., 1998; Chatterjee et al., 1998a), the action is probably via

inhibition of the reuptake of serotonin, norepinephrine and dopamine. In other

words, by preventing the brain from reabsorbing the neurotransmitters in

question, thus, keeping serotonin, norepinephrine and dopamine levels in the

brain at a higher level. It also appears to inhibit reuptake of GABA and L

glutamine, which are the primary inhibitory neurotransmitters in the brain

(Chatterjee et al., 1998a). Very recently, Singer et al. (1999) attempted to

characterize the mechanism of serotonin reuptake inhibition using kinetic

analyses in synaptosomes of mouse brain. Their findings show that hyperforin

inhibits serotonin uptake by elevating free intracellular sodium.

Another component of St. John's wort believed to have antidepressant

effects is the flavonoid amentoflavone (Nahrstedt and Butterweck, 1997;

Baureithel et al., 1997). Recently, Baureithel et al. (1997) suggested that

amentoflavone exerts its antidepressant action by binding benzodiazepine

receptors in the brain.

1.4.2. Antiviral Activity

In vitro, St. John's wort and/or hypericin have been shown to possess

antiviral activity against several viruses including HIV, influenza, herpes and

equine infectious anemia virus (Meruelo et al., 1988; Lopez-Bazzocchi et al.,

1991; Lavie et al., 1994, 1995; Upton, 1997; Cracchiolo, 1998; Gulick et al.,

1999). Recent research has focused on the use of hypericin for the treatment

of AIDS. There have been at least two AIDS clinical trial group studies that

used synthetic hypericin as a treatment for AIDS, and both trials appear to

have had difficulties with photosensitivity reactions (Chavez and Chavez,

1997). A Phase I/II clinical trial in Thailand found that hypericin administered

once a day for 28 days to 12 HIV-infected patients at a dose of 0.05 mg/kg,

resulted in 10 of the 12 patients exhibiting reduced viral load (Chavez and

Chavez, 1997).

However, a recent Phase I study with 30 HIV-infected simplex 1 and 2,

Sindhis virus, murine cytomegalovirus, para-influenza 3 virus and vesicular

stomatitis virus patients with CD4 counts less than 350 cells/mm 3 showed that

intervention with intravenous hypericin, 0.25 or 0.5 mg/kg of body weight

twice weekly or 0.25 mg/kg three times weekly or oral hypericin, 0.5 mg/kg

daily, caused significant phototoxicity and had no antiretroviral activity in the

patients studied (Gulick et al., 1999). Severe coetaneous phototoxicity was

observed in 48% of the patients.

1.4.3. Antibacterial and Antifungal Activity

Several studies with St. John's wort extracts have demonstrated their anti

bacterial and antifungal activities. For instance, two Russian preparations

of Hypericum were found to be more effective than sulfonilamide against

Staphylococcus aureus infection in vivo and in vitro (Derbentseva and Rabin

ovich, 1968; Aizenman, 1969; Gurevich et al., 1971; Hobbs, 1989). The

phloroglucinol derivative hyperforin is known to be an important antibiotic

constituent of Hypericum and is believed to be the agent that inhibits S. aureus

(Negrash and Pochinok, 1972; Brondz et al., 1983). Recently, Rocha et al.

(1995) reported on antibacterial phloroglucinols and flavonoids from H. bra

siliense. A resin fraction of the methanol extract of St. John's wort LI 160

has been shown to have antifungal activity and significant action against gram

positive bacteria (Upton, 1997). Tannins and flavonoids in St. John's wort

have been reported to inactivate Escherichia coli at dilutions of 1:400 and

1:200 (Upton, 1997). Essential oil from H. perforatum also displays antimicro

bial activity (Hobbs, 1989).

1.4.4. Anti-Inflammatory Activity

Preparations of Hypericum have been found to suppress inflammation in vivo

(Hobbs, 1989; Anonymous, 1998a). The likely phytochemicals responsible for

this action are the flavonoids, which constitute 11.7% of the flowers and 7.4%

of the stems and leaves (Lietti et al., 1976; Middleton and Kandaswami, 1992;

Upton, 1997). The mechanisms for the anti-inflammatory activity of flavonoids

include inhibition of arachidonic acid metabolism (Ferrandiz and Alcaraz,

1991) and inhibition of the prostaglandin synthase cyclooxygenase activity

(Hoult et al., 1994). The property of flavonoids to decrease the fragility and

permeability of blood capillaries was originally identified by Albert Szent

Gyorgyi, who coined the term Vitamin P for those compounds that reduce

capillary permeability.

Several prescription and nonprescription pharmaceutical products con

taining flavonoids as the active principle are used to control capillary perme

ability and fragility (Lietti et al., 1976). Clinical applications include treatment

of visual disorders related to nighttime visual acuity, to aid in adapting to low

light conditions, and to decrease recovery time after exposure to glare.

1.4.5. Antioxidant Activity

Antioxidant activity is one of the most important mechanisms for preventing

or delaying the onset of major degenerative diseases of aging, including

cancer, heart disease, cataracts and cognitive dysfunction. The antioxidants

are believed to exert their effects by blocking oxidative processes and free

radicals that contribute to the causation of these chronic diseases (Ames, 1983;

Block, 1992). Several phenolics, known to be present in Hypericum, especially

catechins, flavonols and tannins have been shown to perform these functions

(Block, 1992; Mackerras, 1995; Bors et al., 1996; Mazza, 1997). It has recently

been reported that St. John's wort extracts exhibit antioxidant activity (Anony

mous, 1997).

1.4.6. Wound-Healing Activity

In a number of studies, St. John's wort extracts have

demonstrated wound

healing activity. For instance, a 1975 patent from Germany claims that an

ointment containing an extract of St. John's wort flowers shortened healing

time of burns and showed antiseptic activity (Saljic, 1975). According to the

report, first degree burns healed in 48 hours when treated with the ointment,

while second and third degree burns healed three times faster than burns

treated by conventional methods. St. John's wort has also been compared with

Calendula, another herb commonly used to heal wounds, and according to

Rao et al. (1991), St. John's wort applied topically was found to be more

effective than Calendula. The effects of St. John's wort on wounds and burns

are probably due to the anti-inflammatory and the antibacterial and antifungal

effects mentioned above. It has also been speculated that the wound-healing

properties attributed to Hypericum may be due to the high content of tannins

in the plant/extracts that act as an astringent and have the ability to complex

and precipitate proteins (Chavez and Chavez, 1997). St. John's Wort

1.4.7. Other Effects

Other reported effects of St. John's wort include enhancement of coronary

flow, inhibition of receptor tyrosine kinase activity,

inhibition of release of

arachidonic acid and leukotriene B4 and increase in the production of nocturnal

melatonin (Upton, 1997). One double-blind, placebo-controlled study, con

ducted with 12 older, healthy volunteers, has found that Hypericum extract

LI 160 (300 mg three times daily) increased deep sleep and slightly decreased

REM sleep (Schulz and Jobert, 1994).

1.5. SAFETY

St. John's wort is well known for its ability to cause photosensitivity to

grazing animals, particularly cattle, sheep, horses and goats, and also rabbits

and rats (Hobbs, 1989; Wichtl, 1994; Chavez and Chavez, 1997; Upton, 1997).

Photosensitivity in livestock is referred to as hypericism or "light sickness"

(Bombardelli and Morazzoni, 1995). Reported reactions are mainly dermato

logical, such as severe erythema and edema of skin, conjunctiva and bucal

mucous membranes, which can lead to restlessness, psychomotor excitement

blindness and refusal to eat by the animals (Araya and Ford, 1981). Because

of refusal to eat and loss of appetite, the threat of Hypericum intoxication to

livestock is reduced, which makes the absorption of the photodynamic pigment,

hypericin, self-limiting (Araya and Ford, 1981).

Phototoxicity in humans appears to be rare; most authors express no concerns

(Hobbs, 1989; Bombardelli and Morazzoni, 1995). This viewpoint is supported

by the results of a recent placebo-controlled randomized clinical trial in which

13 volunteers received 900, 1,800 or 3,600 mg of a standardized Hypericum

extract (LI 160) containing zero, 2.81, 5.62 and 11.25 mg of total hypericin

(total hypericin is the sum of hypericin and pseudohypericin) (Brockmoller

et al., 1997). Nonetheless, most authors recommend caution when using large

quantities of St. John's wort extract for therapeutic uses, particularly for people

with fair skin, who should not expose themselves to strong sunlight during

Hypericum therapy (Hobbs, 1989; Bombardelli and Morazzoni, 1995).

Recently, a systematic review on adverse effects of H. perforatum by

Rand et al. (1998) concluded that St. John's wort is well tolerated. It has an

encouraging safety profile with an incidence of adverse reactions similar to that

of placebo. The most common adverse effects are gastrointestinal symptoms,

dizziness/confusion and tiredness/sedation. Photosensitivity appears to occur

extremely rarely. Similarly, in a recent comparative trial, 63% of patients

receiving St. John's wort reported that they experienced no adverse effects

during treatment. In contrast, only 36% of amitriptyline-treated patients re

ported no adverse effects. Hypericum has also been associated with signifi

cantly less dry mouth and drowsiness than amitriptyline (Wheatley, 1998).

Similar results were reported by Stevinson and Edzard (1999) in a compari

son of St. John's wort with the conventional antidepressants dothiepin, fluoxe

tine, moclobemide and mirtazapine. In this study, the authors also found that

Hypericum was associated with fewer and milder adverse drug reactions in

clinical trials than any of the other drugs. Data on the safety of Hypericum

in overdose and on interactions with other drugs are as yet scarce.

1.6. CONCLUSIONS

Extracts of the plant H. perforatum have been used in herbal medicine for

centuries. Traditional uses include treatment of nervous disorders, insomnia,

burns, shocks, hysteria, gastritis, hemorrhoids, urinary disorders, scabies and

wounds. Currently, St. John's wort is best known for the treatment of mild

to moderate depression. The constituent of Hypericum extracts responsible

for the antidepressant action is, however, unknown, and does not appear to

be hypericin, the constituent by which the extracts are currently standardized.

Based upon limited studies, St. John's wort appears to be an acceptable

alternative to traditional antidepressant therapy. In the 14 double-blind pla

cebo-controlled trials that have been performed to date, 55% of patients receiv

ing Hypericum were classified as responders (defined as those patients showing

a 50% reduction in the severity of depression from baseline), compared with

22% of patients receiving placebo. In similar comparative trials, Hypericum has

been shown to be as effective as standard tricyclic antidepressants (imipramine,

amitriptyline and maprotiline). Data indicate that Hypericum is a well-tolerated

alternative to synthetic drugs for the treatment of mi Id-to-moderate depression,

particularly in patients who are intolerant of standard antidepressants. Trials

comparing the effect of St. John's wort with selective serotonin (5-hydroxy

tryptamine; 5-HT) reuptake inhibitors and other newer antidepressants and

assessing the effect of higher dosages in patients with severe depression are

required to fully determine the place of Hypericum in the treatment of de

pressive illness.

In vitro investigations of Hypericum show antiviral activity, although there

is evidence that these promising results might not occur in vivo. Photosensitiv

ity has been reported in animals that have eaten large quantities of Hypericum,

however, no cases of photosensitivity have been reported in humans.

2. GINKGO BILOBA

2.1. INTRODUCTION

Ginkgo {Ginkgo biloba L.), also known as maidenhair-tree, of the Ginhgo

acea family, is a dioecious tree reaching up to 30 m tall, with a bole circumferGinkgo Biloba 147

ence of up to 9 m, and is believed to be native to China. The name of the

genus Ginkgo is derived from a mistranslation of the Japanese name, Yin

Kwo, meaning "silver fruit." The leaves are deciduous, alternate or in clusters

of three to five on short twigs, petiole, fan, thickened at the margins, 5-10

cm across and bilobed, hence, the species biloba appellation. The seed is

yellow, round, about 2.5 cm long, with bad-smelling pulp surrounding the

thin-shelled white nut that contains an edible sweet kernel.

The leaf extract of Ginkgo biloba is one of the oldest natural therapeutic

agents still used today. The Ginkgo biloba tree has long been part of the

traditional Chinese pharmacopeia, first cited as a medicinal agent about 5,000

years ago (Michel, 1986). It was first cited in Chinese herbâis around the

fourteenth century AD for its "fruit" that was consumed raw

or cooked. In

traditional Chinese medicine, Ginkgo seeds are prescribed as a remedy against

asthma, cough, bladder inflammation, Menorrhagia and alcohol abuse (Chang

and But, 1987). Anticarcinogenic and vermifugal properties have also been

claimed for the raw nuts. The medicinal uses of Ginkgo leaves in cardiovascular

disorders and asthma dates back to 1550 according to traditional Chinese

medicine. At present, extracts of Ginkgo leaves are used extensively for

treatment of memory disorders associated with aging, including Alzheimer's

disease and vascular dementia. In the United States, nearly 11 million people

took Ginkgo extract in 1997 (Giese, 1999). The physiological benefits of

Ginkgo are generally attributed to the antioxidant, vasoregulating and neuro

protective properties of some of its constituents.

2.2. CHEMICAL COMPOSITION AND CHEMISTRY

Ginkgo contains numerous compounds with documented biological activity.

Constituents that have been most studied include the ginkgolides, flavonoids

and ginkgolic acids.

2.2.1. Ginkgolides

2.2.1.1. Structural Characteristics

Ginkgolides are molecules that only occur naturally in the leaves and roots

of Ginkgo biloba. They were first isolated in 1932 from the bitter principles

of Ginkgo biloba, but their structure was only resolved in 1967 (Furukawa,

1932; Maruyama et al., 1967a). In a series of studies, Maruyama et al. (1967b,

1967c, 1967d) characterized the ginkgolides as 20 carbon cage molecules,

incorporating a t-butyl group and six, five-membered rings A to F including

a spironane, a tetrahydrofuran cycle and three lactone rings. The ginkgolides

possess three lactone rings and differ only in the number and positions of

substitutes. Four structures were identified differing only by the number and CHEMISTRY, PHARMACOLOGY AND CLINICAL APPLICATIONS Figure 6.4 Structure of ginkgolides.

position of hydroxy 1 groups on the Cl , C3 or CI of the spironane framework.

These compounds were named ginkgolides A, B, C, M and J (Figure 6.4).

Termed BN 52020, BN 52021, BN 52022, BN 52023 and BN 52024, respec

tively, in Institut Henri Beaufour (IHB) internal nomenclature, these represent

the four platelet activating factor (PAF) antagonists initially characterized in

Ginkgo biloba leaf extract EGb 761.

Ginkgoiide B (BN 52021), the most active PAF antagonist found in this

class, is a specific and potent competitive antagonist of the binding of PAF

to its membrane receptor in human platelet and lung

preparations. Ginkgolides

A (BN 52020) and C (BN 52022) are about one order of magnitude less potent

than BN 52021, the less active compound being BN 52022. The PAF antagonist

activity of ginkgoiide B is significantly reinforced by combining it with gink

golides A and C. BN 52063, a standardized mixture of ginkgolides A, B and

C (in the ratio 2:2:1) is the first drug that has been shown to be a potent PAF

antagonist in humans (Guinot et al., 1988).

Ginkgolides are derived from one of the most complex natural molecular

frameworks in which mevalonate and methionine are incorporated. The gink

goiide structure bears a striking resemblance to that of bilobalide, another Ginkgo Biloba

constituent of Ginkgo biloba. This incorporates a tertbutyl group and five

fused cyclopentanoid rings, among which are again three 7-lactone (but no

tetrahydrofuran) and only one cyclopentane and, hence, no spiro units.

2.2.1.2. Occurrence and Pathophysiology

Ginkgolides have been extracted from various organs of the Ginkgo tree.

They are found in the leaves, roots and root bark. The biosynthesis of gink

golides occurs at a very early stage in the development of the tree. Because

leaves are a renewable resource that can be harvested every year, it is the

organ of choice for large-scale extraction of ginkgolides.

The best source of the ginkgolides is the root bark, yielding 0.01 % ginkgolide

A (GA), 0.01% ginkgolide B (GB), 0.02% ginkgolide C (GC) and 0.0002%

GM after extraction, chromatography and multiple (10-15 steps) fractional

recrystallization steps (Nakanishi, 1988).

Seasonal variations in ginkgolide content of leaves have been observed. It

reaches a maximu m in late summer and falls to a minimum at the end of

autumn. There are also variations in the ratios of individual ginkgolides with

each other in leaves from different geographical origins that may be attributed

to variation in climate, conditions of culture and differences within species.

According to Braquet (1988), ginkgolides have therapeutic effects in differ

ent pathophysiological processes and models of disease, such as inflammation,

airway hyperactivity, the cardiovascular system, endotoxemia and other mod

els of shock, gastrointestinal ulceration, the renal system, CNS functions,

immune processes, ocular diseases and skin diseases (Table 6.3).

Since a potential role for PAF has been ascribed in various pathologies

such as inflammation, allergy, sepsis or thrombosis, the ginkgolides might be

of therapeuti c interest, not only by inhibiting the direct effects of PAF such

as lung tissue contraction, vascular tone increase or aggregation, but also by

blocking the formation of the other mediators, thromboxane A2 or leukotrienes.

In addition, the role of PAF in late asthmatic responses could trigger the

recruitment of inflammatory cells such as neutrophils, macrophages/monocytes

and eosinophils seen in bronchial tissue from asthmatic patients. Ginkgolides

may thus provide new insight in the treatment of bronchial asthma.

Topical or systematic administration of the ginkgolide BN 52021, a specific

inhibitor of PAF-acether, not only inhibits thrombus formation but equally

prevents recruitment of leukocytes and recurrence of the thrombic phenomenon

attributed to the generation of PAF-like activity in the vessel wall. Since BN

52021 potently inhibits the PAF-acether-induced eosinophil chemotaxis, it

(BN 52021) may be useful in preventing inflammation and in treating bronchial

asthma. BN 52021 is able to block [Ca**] transient in the presence and absence

of extracellular calcium for human platelets. Thus, extract of Ginkgo biloba

leaves (EGb) and its constituent ginkgolide B prevent neuronal damage through TABLE 6.3. Therapeutic Effects of Ginkgolides. Disease Airway hyperactivity Cardiovascular system CNS function Gastrointestinal ulceration Immune process Physiological Response reduces cell inflammation

inhibits weal and flare in human skin inhibits cutaneous response modifies allergic skin responses and allergic asthma inhibits bronchial allergen challenge in humans antagonizes inflammation responses in humans inhibits coronary vasoconstriction inhibits hypotension prevents or reverses pulmonary derangements prevents anaphylactic shock of the heart inhibits vasodilation of mesenteric microvessels mediates noradregenic system improves cerebral metabolism and reverses cerebral impairment alleviates post-stroke syndrome by reducing cerebral edema inhibits deterioration of regional cerebral blood flow caused by injury reduces blood-brain barrier permeability inhibits endotoxic shock reduces endotoxm-induced gastrointestinal leakage reduces endotoxm-induced impairment in stomach and small intestine ameliorates mucosal damage associated with the small intestine regulates immune reactivity modulates immune response prevents suppression of deleterious immunological reactions suppresses rejection of bone marrow transplants prevents or inhibits cellular rejection prolongs graft survival

0 TABLE 6.3. (continued) Disease Inflammation Leukocytes and endothelial cells Ocular disease Platelets and thrombosis Renal system Shock Skin disease Physiological Response reduces edema blocks inflammatory action inhibits degranulation, superoxide generation and chemotaxis of neutrophils protects viabil ty of endothelium modulates physico-chemical state of cytoskeleton via calcium increases vascular permeability protects retinal tissue against argon laser damage improves healing of corneal wounds and decreases corneal swelling modulates treatment of retinal functional impairments inhibits inflammatory response of the anterior segments of the eye antagonizes platelet binding interferes with fibrinogen binding and aggregation prevents the activation of transmembrane events blocks the formation of several mediators (thromboxane A 2 and leukotnenes) protects tissue from post-ischemia oxidative damage blocks glomerular filtration and urinary sodium excretion reverses glomeruli and mesangial cell extraction affords protection against renal damage interferes with changes in blood pressure restores the capability of lung parenchymal strips prevents increase of vascular permeability prevents arterial hypotension inhibits intestinal lesions induced by endotoxins induces progressive increase in glycemia inhibits the release of postaglandins inhibits candida killing activity of human keratinocytes suppresses allergic dermatitis reduces swelling of cutaneous tissues during contact dermatitis

Compiled from Braquet (1988).

reduction of the rise in [Ca 2+]I (Zhu et al., 1997). BN 52021 (20 mg/kg) given

orally one hour before the application of a skin test had a small but significant

effect on inhibiting the late phase obstruction airway response. The PAF

antagonist BN 52021 has no effect on the pulmonary or systemic circulation

per se. However, it completely blocks the increase in pulmonary arterial

pressure and vascular resistance as well as the decrease in cardiac output

produced by PAF. It seems that BN 52021 is especially potent in blocking

the renal receptor of PAF. At low doses (< 25 |xg/kg), BN 52021 efficiently

blocks the effect of PAF on glomerular filtration in dogs. In the dog, ginkgolide

B inhibits the decrease of the glomerular filtration rate and urinary sodium

excretion induced by PAF injection.

BN 52063, an extract of ginkgolides containing a mixture of BN 52020,

BN 52021 and BN 52022, has been shown to be a selective PAF-antagonist

in humans. It inhibits PAF-induced weal and flare in human skin while it has

no effect on histamine-induced weal and flare responses (Page and Robertson,

1988). BN 52063 dose-dependently inhibits the coronary vasoconstriction

caused by PAF-acether. It also elicits a decrease in heart rate with a maximum

effect after 2 min. In humans, BN 52063 has been shown to inhibit PAF

induced skin plasma exudation and platelet aggregation.

Ginkgolides significantly inhibit PAF-acether effects and various immune

disorders both in vivo and in vitro in humans and in animals. BN 52021, the

most potent of ginkgolide, inhibits PAF-binding to rabbit- and human-washed

platelets, PAF-induced aggregation and degranulation of human isolated neu

trophils, PAF-induced contraction of guinea pig lung parenchymal strips or

IgG-induced hypotension, hemo concentration and extravasation in the rat.

BN 52021 also antagonizes edema, cell filtration, eicosanoid release and lipid

peroxidation of intestine and heart ischemia (Spinnewyn et al., 1988).

2.2.2. Bilobalide

Bilobalide is a sesquiterpene lactone believed to be a degraded ginkgolide

(loss of carbon 2, 3, 14, 15 and 16) (Nakanishi, 1988). Bilobalide is unstable

under basic conditions and may play a significant role in protecting Ginkgo

biloba against herbivorous insects or mammals.

Chatterjee et al. (1986) indicated that bilobalide-containing agents might

provide therapy for neurological disorders that are caused

by, or associated

with, pathological changes in the myelin sheaths of nerve fibers. In pharmaco

logical models, bilobalide is thought to posses anti-edematous, astrocyte

stimulating and myelin-protecting effects (Chatterjee et al., 1986). Bilobalide

has also been found to be suitable for the treatment of certain nervous diseases

such as neuropathies, encephalopathies and myelopathies in animal models

(Chatterjee et al., 1990).

Bilobalide, as well as ginkgolide B, are known to protect cultured neurons

from hypoxia- and glutamate-induced damage. Recently, Ahlemeyer et al. Ginkgo Biloba

(1999) reported that among the constituents of Ginkgo biloba extract, bilobal

ide has the most potent anti-apoptotic capacity.

2.2.3. Flavonoids

Ginkgo-ñavone glycosides are mono-, di- or tri-glycosides whose aglycon

is a flavonol (quercetin, kaempferol or isorhamnetin) and whose glycosidic

constituents are glucose and rhamnose. Different classes of flavonoids, includ

ing dimeric flavonoids, flavonols, flavonol glycosides and coumaric esters of

flavonol glycosides have been isolated from Ginkgo biloba leaves (Van Beek

et al., 1998). The biflavones, amentoflavone, bilobetin, sequojaflavone, gink

getin, isoginkgetin and sciadopitysin are present in small amounts but are of

major therapeutic interest. The biflavone ginkgetin strongly reduces arthritic

inflammation in the rat and may, therefore, be a potential antiarthritic agent

with analgesic activities (Kim et al., 1999). The structure and chemistry of

these biflavones together with that of a I-5'-methoxybilobetin have been eluci

dated (Joly et al., 1980) (Figure 6.3).

Flavonoids isolated from commercial extract (EGb 761) taken at a dose of

80 mg, three times a day in a placebo-controlled double-blind study with

quadruple crossover design in 12 participants showed cerebral electrical activ

ity in the frontal region suggesting that the flavonoids were bioavailable to

the brain (DeFeudis, 1991). The antithrombotic and vasoregulatory activities

of commercial Ginkgo extracts have been attributed to the antioxidant and

oxygen scavenging activities of the flavonoid constituent of these extracts.

Two flavonols, quercetin and rutin, present in Ginkgo biloba leaves are

potent antithrombotic agents in vivo, and hence, it follows that these flavonoids

and their glycosides could be relevant constituents responsible for the thrombo

lytic and vasoprotective actions of commercial extracts observed in clinical

settings (DeFeudis, 1991). In addition, quercetin and kaempferol have the

same hydrogen-donating antioxidant mechanism as Ginkgo biloba extract (Shi

and Niki, 1998). These flavonols consumed in the form of Ginkgo biloba

tablets are eliminated in the human urine as glucuronides (Watson and Oliveira,

1999). A mixture of flavonols (quercetin, kaempferol, isorhamnetin) extracted

from Ginkgo biloba leaves has been shown to inhibit contraction of isolated

guinea pig intestine and, therefore, may exert inhibitory effects on spasms of

the intestine (Peter et al., 1966).

Flavonoids are also potent inhibitors of cyclic-AMP-phosphodiesterase,

thereby enhancing the effects of prostaglandin 12 or prostacyclin, mediators of

vasoprotection and thrombosis. The degree of cyclic-AMP-phosphodiesterase

inhibition by Ginkgo biloba bioflavones in rat adipose tissue was found to be

amentoflavone > bilobetin > sequoiaflavone > ginkgetin = isoginkgetin, and

sciadopitysin was almost inactive (Saponara and Bosisio, 1998). Amentafla

vone is also known to exhibit five to 10 times greater potency than papaverine

in inhibiting the degradation of cyclic-GMP, which in turn leads to endothe

lium-dependent relaxation of vascular smooth muscle (DeFeudis, 1991).

Quercetin and kaempferol coumaroyl glycosides are found only in Ginkgo

biloba, and the latter is considered particularly essential for the efficacy of

commercial extracts. Some of the flavonol glycosides are unique to Ginkgo

biloba and have, therefore, been used for the standardization of commercial

extracts. Several HPLC methods developed for the qualitative and quantitative

determination of flavonoids of Ginkgo biloba have recently been summarized

(Van Beek et al., 1998). The flavonoid glycosides are generally acid hy

drolyzed, cleaned by solid-phase extraction cartridges or diluted prior to quanti

tatively determining the corresponding aglycones (quercetin, kaempferol and

isorhamnetin) (Stricher, 1993). Fingerprinting of all Ginkgo biloba flavonoids

using RP-HPLC allows the separation and identification of 33 flavonoids (22

flavonoid glycosides, six flavonoid aglycones and five biflavones) by elution

order and diode-array UV spectra (Van Beek et al., 1998). The bioflavone

constituents of Ginkgo biloba leaves have been identified and confirmed using

HPLC-MS with a thermospray interface (Gobbato et al., 1996).

Rutin and quercetin as well as mixtures of flavonoids from Ginkgo biloba

extracts protect cerebellar granular cells from oxidative damage and apoptosis

due to the scavenging activity of their hydroxyl radicals (Chen et al., 1999).

The biflavone ginkgetin isolated from Ginkgo biloba leaves may be a potential

antiarthritic agent with analgesic activity. It strongly reduces arthritic inflam

mation and inhibits wreathing in the animal model (Kim et al., 1999). Flavo

noids in Ginkgo biloba extract have been credited with the protection of

endothelial cells against hyperoxia and hypoxia-reoxygenation due to their

antioxidant activity. These protective effects of the flavonoids account for the

wide use of Ginkgo biloba extracts in venous diseases (Remade et al., 1990).

2.2.4. GinkgoWc Acids

The chemistry and biology of ginkgolic acid and related alkyphenols from

Ginkgo biloba have recently been reviewed (Jaggy and Koch, 1997). Ginkgolic

acids are 6-alkyl salicylic acids and are the major components of the lipid

fraction in the nutshells of Ginkgo biloba. This class of substances is also

present in Ginkgo leaves. The 3-alkyl phenol is called ginkgol and the 5

alkyl resorcins, derived via decarboxylation of 6-alkyl resorcylic acids, are

represented by bilobol and hydrobilobol (Figure 6.5). The simple unsaturated

C15-ginkgolic acid is the main component of the nutshells and leaves (3.1 and

1.2%, respectively), followed by C17:1-acid (0.22 and 0.44%, respectively). A

ginkgolic acid content of 1.73% was determined in dried Ginkgo biloba leaves

by HPLC.

Contact with Ginkgo biloba fruit is frequently reported to induce allergic

skin reactions generally attributed to the presence of ginkgolic acids. The Ginkgo Biloba Figure 6.5 Structure of ginkgolic acids, cardanols and cardols.

alkylphenolic acids are also known to cause gastrointestinal disturbances fol

lowing the consumption of Ginkgo biloba fruit (Becker and Skipworth, 1975).

The allergenic properties of alkylphenols are presumably based on their chemi

cal structure, which makes them capable of being incorporated in cell mem

branes. Ginkgolic acid has immunotoxic properties and causes swelling of the

lymph nodes in mice (Jaggy and Koch, 1997). It has very high sensitizing

potential even at very low concentrations (125 |xg ginkgolic acids). Ginkgolic

acids have several biological activities, including dehydrogenase inhibitory

activity, antimicrobial activity against Mycobacterium smegmatis, Bacillus

subtilis and Staphylococcus aureus, molluscicidal activity, antitumoral activity

and an inhibitory effect on seed germination (Jaggy and Koch, 1997). Ginkgolic

acids are effective inhibitors of glycerol-3-phosphate dehydrogenase, a key

enzyme in the synthesis of triacylglycerol (Irie et al., 1996). Inhibition of

glycerol-3-phosphate dehydrogenase may lead to low accumulation of lipids

resulting in reduced risk of various diseases such as hyperglycemia, diabetes,

myocardial infarction and high blood pressure. It has been speculated that the

alkylphenols contribute significantly to the high resistance of Ginkgo biloba

to different adverse environmental influences.

2.2.5. Other Constituents

A lipid fraction (5% total lipids) extracted from Ginkgo seed contains 4

hydroxyanacardic acids (97%), precursors of 5-alkylresorcinol. The lipids of

immature Ginkgo seeds contain up to 75% anacardic acids that have proven

antibiotic properties (Gellerman et al., 1976).

2.3. COMMERCIAL PREPARATIONS

Extracts from Ginkgo biloba are prepared from dried leaves using acetone/

water and subsequent purification and concentration steps aimed at increasing

the concentration of bioactive phytochemicals (Anonymous, 1998b; DeFeudis,

1991; Kleijnen and Knipschild, 1990). An extract of Ginkgo biloba leaves

was first registered in Germany in 1965. It was introduced in the market under

the Tebonin® trade name as drops, tablets and later as ampoules. Development

of a highly purified extract at single doses of 40 mg led to the registration

in 1974 of the oral preparation under the trade name Tanakan® in France.

Preparations of this extract were introduced in Germany as Rokan® in 1978

and as Tebonin® forte in 1982. The extract obtained the code name EGb 761

(Extractum Ginkgo bilobae 761) and is currently marketed in over 30 countries

under different trade names (DeFeudis, 1991). EGb 761 is the most studied

and commonly used Ginkgo preparation in clinical trials.

The pharmacological activities and clinical applications of Ginkgo biloba

extract EGb 761 have been extensively reviewed by DeFeudis (1991) and

more recently by Clostre (1999). The extract is standardized on the amount

of ginkgo-flavone glycosides (24%) and terpenoids (ginkgolides A, B, C, J

and bilobalide) (6%). Ginkgolides A, B and C, collectively account for about

3.1%, ginkgolide J <0.5% and bilobalide about 2.9% of EGb 761 extract.

Organic acids account for about 5-10% of EGb 761 and give the extract an

acidic character, thereby increasing its water solubility (DeFeudis, 1991).

Another Ginkgo preparation often encountered in clinical trial is Kaveri (also

called LI 1370) that is standardized on the same ingredients in doses similar

to EGb 761 (25% ginkgo-flavone glycosides, and recently, also 6% terpenoids)

(Kleijnen and Knipschild, 1990). The composition of Ginkgo preparations

may vary depending on the manufacturing process used, and hence, different

effects can result. More than 24 different brands of Ginkgo biloba extract are

sold in the United States.

2.4. PHARMACOLOGICAL EFFECTS

Currently, Ginkgo is widely used in Europe for the treatment of memory

disorders associated with aging, including Alzheimer's disease (AD) and vas

cular dementia. Over 5 million prescriptions were issued mostly by physicians

for Ginkgo in 1988 in Germany (Oken et al., 1998). In 1994, a standardized

dry extract of Ginkgo biloba leaves (SeGb) was approved by German health

authorities for the treatment of primary degenerative dementia and vascular

dementia. Ginkgo biloba extract is already widely used in the United States

as an alternative therapy for AD. The wide use of Ginkgo biloba extracts is

based on the pharmacological action resulting from the combined activities

of several active principles (primarily, the flavonoids and terpenoids). This

"polyvalent" (terminology used by DeFeudis, 1991) action

explains the vaso

and tissue-protective and the cognition-enhancing benefits of the Ginkgo biloba

extract (Van Beek et al., 1998).

2.4.1. Clinical Trials

The principal clinical trials concerning the therapeutic applications of

Ginkgo biloba extract have been extensively reviewed (Braquet, 1988; De

Feudis, 1991; Van Beek et al., 1998; Kleijnen and Knipschild, 1990, 1992).

According to these reviews, the therapeutic effect of Ginkgo biloba can be

generally attributed to its antioxidant, anti-ischemic, cardioprotective and va

soregulating activities and neuroprotective properties. The pharmacological

studies on the Ginkgo biloba extract are still ongoing. Several new clinically

controlled studies confirming the efficacy of Ginkgo biloba extract in important

pathologies have appeared since the publications of the above cited reviews

and are summarized in Table 6.4 and briefly discussed here.

Chung et al. (1999) recently evaluated a possible therapeutic effect of

Ginkgo biloba extract (GBE) on glaucoma patients that may benefit from

improvements in ocular blood flow. A Phase I crossover trial of GBE with

placebo control in 11 healthy volunteers (eight women, three men: age, 34

± 3 years, mean ± SE) was performed. Patients were treated with either GBE

40 mg or placebo three times daily, orally, for two days. Color Doppler

imaging (Siemens Quantum 2000) was used to measure ocular blood flow

before and after treatment. There was a two-week washout period between

GBE and placebo treatment. Ginkgo biloba extract significantly increased end

diastolic velocity (EDV) in the ophthalmic artery (OA) (baseline vs. GBE

treatment; 6.5 ± 0.5 vs. 7.7 ± 0.5 cm/s, 23% change, p = 0.023), with no

change seen in placebo (baseline vs. GBE treatment: 7.2 ± 0.6 vs. TABLE 6.4. Recent Clinical Studies with Ginkgo biloba Extract. Authors Chung et al., 1999 Janssens et al., 1999 Lingaerde et al., 1999 Rigney et al., 1999 Castelli et al., 1998 Type of Study Phase I crossover trial Randomized double-blind Randomized double-blind Randomized double-blind Double-blind vs. placebo No. Patients 11 (8 w/3 m) — 27 Diagnosis Glaucoma Patients with primary chronic venous insufficiency SAD (seasonal affective disorder) 31 (ages 30-59) Asymptomatic 22 (women of 22-55 yrs) volunteers Allergic contact dermatitis Dosage and Duration 40 mg, 3X daily for two days 4 wk treatment with Ginkor Fort PN246 Tablet 10 wks 120-300 mg (50-100 mg i.d.s.) for two days GBE and sodium carboxy methyl-beta1,3-glucan applied to intact skin 2X a day for 2 wks prior to contact allergen Improvement Increased end diastolic velocity in ophthalmic artery 23/3% change % decrease in circulating endothelial cell count 14.5/8.4 No significant improvement in symptoms of winter depression GBE 120 mg pronounced improvement in aspects of cognition 68% reduction in skin reactivity of treated vs. placebo Overall Assessment Increase in ocular blood flow Beneficial action of GBE on venous wall Development of the symptoms of winter depression not preventable by GB Cognitive enhancing effects of GBE likely more apparent in 50-59 yr individuals GBE formulation mitigates allergic contact dermatitis

8 TABLE 6.4. (continued) Authors Cesarani et al., 1998 Ivaniv, 1998 Li et al., 1998 Li et al., 1997 Type of Study Open, controlled vs. placebo — Controlled trial Randomized controlled trial No. Patients 44 208 24(12/12, diabetic/ nondiabetic) — Dosage and Diagnosis Duration Vertiginous syndrome 80 mg, 2X daily, three months Discirculatory — encephalopathy (DE) Peripheral arterial 240 mg • d' 11 , occlusive disease PO, 48 wks (PAOD) Asthma — Improvement Improved viscovestibular ocular reflex Positive changes in cognitive behavior Pain-free walking distance 3.3-3.8fold increase Reduced airway hyperreactivity Overall Assessment Beneficial action of G BE in treatment of equilibrium disorders Positive in rehabilitation of patients affected with DE Therapeutic effect in PAOD patients Improved symptoms of pulmonary functions G BE = Ginkgo biloba extract.

1 5 9

7.1± 0.5 cm/s, 3% change, p = 0.892). No side effects related to GBE were

found. Ginkgo biloba extract did not alter arterial blood pressure, heart rate

or IOP. Since Ginkgo biloba extract significantly increased EDV in the OA,

Chung et al. (1999) suggest that GBE may possibly find application in the

treatment of glaucomatous optic neuropathy and other ischemic ocular dis

eases.

One possible mechanism that accounts for the alterations observed in vari

cose veins is the activation of endothelial cells by ischemia occurring in the

leg veins during blood stasis and the cascade of reactions that follows. Because

in vitro data suggest that endothelium alteration is a key event in the develop

ment of the pathology, Janssens et al. (1999) conducted a clinical trial to

confirm this hypothesis. The authors used the number of circulating endothelial

cells (CECs) detached from the vascular wall as a criterion of the endothelium

injury in patients with chronic venous insufficiency (CVI) and determined the

protective effect of Ginkgo biloba extract (Ginkor Fort), by a randomized,

double-blind, placebo-controlled clinical trial. Their results showed that in the

active-treatment group, the mean values of the CEC count decreased by 14.5%

after a four-week treatment, whereas in the placebo group, the decrease was

less (8.4%).

Ginkgo biloba extract PN246, in tablet form (brand name Bio-Biloba) has

also been used to test the hypothesis that it may prevent the symptoms of

winter depression (WD) in patients with seasonal affective disorder (SAD)

(Lingaerde et al., 1999). In this study, a total of 27 SAD patients were

randomized to receive double-blind placebo or Bio-Biloba for 10 weeks or

until they developed symptoms of WD, starting in a symptom-free phase about

one month before expected WD symptoms. An extended Montgomery-Asberg

Depression Rating Scale was completed before and immediately after termina

tion of medication. The patients also self-rated some key symptoms on a

visual analogue scale every two weeks during the trial. Since differences

between the treatment groups in the number of patients who developed treat

ment-requiring WD, or in the development of single key symptoms during

the trial were not significant, it was concluded that Ginkgo biloba was unable

to prevent the development of the symptoms of winter depression.

A study on the effects of acute doses of a Ginkgo biloba extract (GBE) on

memory and psychomotor performance in a randomized, double-blind and

placebo-controlled five-way crossover design was carried out by Rigney et

al. (1999). In this study, 31 volunteers aged 30-59 years received GBE 150

mg (50 mg t.d.s.), GBE 300 mg (100 mg t.d.s.), GBE 120 mg mane and GBE

240 mg mane and placebo for two days. Following baseline measures, the

medication was administered at 0900 h for the single doses and at 0900,

1500 and 2100 h for the multiple doses. The psychometric test battery was

administered pre-dose (0830 h) and then at frequent intervals until 11 h post

dose. The results demonstrate pronounced effects of GBE extract on aspects

of cognition in asymptomatic volunteers for memory,

particularly working

memory. They also show that these effects may be dose dependent though

not in a linear dose-related manner, and that GBE 120 mg produces the most

evident effects of the doses examined. Additionally, the results suggest that

the cognitive enhancing effects of GBE are more likely to be apparent in

individuals aged 50-59 years (Rigney et al., 1999).

The clinical efficiency of mitigating contact dermatitis with a Ginkgo biloba

extract and carboxymethyl-beta-1,3-glucan formulation was investigated in a

double-blind versus placebo study using 22 subjects (Caucasian women aged

22-55 years) with allergic contact dermatitis from various substances in the

European standard series (Castelli et al., 1998). The formulation was applied

to intact skin two times a day for two weeks ("in use" application) prior to

a single application of a selected contact allergen under a Finn Chamber for

24 h. Readings were carried out in a blind study by a dermatologist two and

three days after patch removal. Representative photographs were taken of

treated, placebo and untreated test areas. Significantly reduced skin reactivity

was shown by 68.2% of the panelists (p = 0.037) on the treated site two days

after patch removal, versus untreated and/or placebo sites.

Thus, according to

Castelli et al. (1998), Ginkgo biloba/carboxymethyl-beta-1,3-glucan formula

tion can mitigate against allergic contact dermatitis.

In an open, controlled study (Cesarani et al., 1998), 44 patients complaining

of vertigo, dizziness or both, caused by vascular vestibular disorders were

randomly treated with an extract of Ginkgo biloba (EGb 761), 80 mg twice

daily, or with betahistine dihydrochloride, (BI) 16 mg twice daily, for three

months. A complete neuro-otologic and equilibrimetric examination was per

formed at baseline and after three months of treatment, with evaluation of

clinical findings. In the first month of therapy, vertigo and dizziness improved

in 64.7% of patients treated with BI and in 65% of those who received EGb

761. Compared to baseline, no statistically significant changes were observed

in cranial scans for patients with a "central" cranial pattern and for the equilib

rium score in both groups. The comprehensive test battery showed that EGb

761 considerably improved visuovestibular ocular reflex. No side effects were

recorded during the trial except for transient mild headache and gastric upset

in two patients receiving EGb 761 and transient cyanosis of nails and lips in

one patient given BI. These results suggest that EGb 761

and BI operate at

different equilibrium receptor sites and show that EGb 761 can considerably

improve oculomotor and visuovestibular functions.

In an open trial, Ginkgo biloba extract was found to be 84% effective in

treating antidepressant-induced sexual dysfunction predominately caused by

selective serotonin reuptake inhibitors (SSRIs, n = 63) (Cohen and Bartlik,

1998). Women (n = 33) were more responsive to the sexually enhancing

effects of Ginkgo biloba than men (n = 30), with relative success rates of

91% versus 76%. Ginkgo biloba generally had a positive effect on all four

phases of the sexual response cycle: desire, excitement (erection and lubrica

tion), orgasm and resolution (afterglow).

This study was later confirmed by another open trial consisting of 55 patients

with sexual dysfunction receiving a standardized Ginkgo extract, 40 or 60 mg

capsules taken three to four times daily (Cohen, 1999). Forty-nine of the

55 patients reported significant response and improvement in their sexual

dysfunction. There were no significant adverse effects associated with the use

of Ginkgo biloba extract, except for one patient who reported worsening of

urinary difficulties related to prostatic hypertrophy (Cohen, 1999). Dry pow

dered extract from leaves of Ginkgo biloba has synergistic effect with lyophy

lized roe and the mixture as such has been used for treating impotence in

human males (Omar, 1998).

Studies made in 208 patients with discirculatory encephalopathy using

clinical and neuropsychological tests, apparatus (REG, ECG, EEG, Doppler

sonography) and laboratory (coagulogram) methods showed the prescription

of drug preparations from Ginkgo to be a worthwhile exercise likely to benefit

the patient during different stages of his/her ailment (Ivaniv, 1998). Ginkgo

preparations showed positive time-related changes in elastic and tonic charac

teristics of vessels, bioelectric brain activity, emotional and behavioral, cogni

tive and mnestic features, especially when combined with a complex of compo

nents of the tricarboxylic acid cycle.

The effects of Ginkgo biloba extract EGb 761 from the points of view of

hemorheology for patients of peripheral arterial occlusive diseases (PAOD)

were studied by Li et al. (1998). The treatment with EGb (240 mg/d, po) and

the pain-free walking distance (PFWD) were carried out for 24 PAOD patients

(12 nondiabetic, ND and 12 diabetic, D) over 48 weeks. The parameters

erythrocyte stiffness (ES) and relaxation time (RT), the blood plasma viscosity

(eta), the plasma fibrinogen concentration (CO and the blood sedimentation rate

(BSR), the PFWD, and maximal walking distance (MWD) were determined at

six weeks before treatment (-6), at the beginning of the treatment (0), and

after 6, 11, 16 and 48 weeks of treatment. At week 0, stiffness and relaxation

time were significantly higher than healthy control, and the mean PFWD was

only 111m. The blood plasma viscosity value was significantly elevated

and fibrinogen concentration and blood sedimentation rate were enhanced.

Throughout 11 weeks of treatment, ES, RT, eta and Cf decreased gradually,

and PFWD improved. Between 16 and 48 weeks, ES and RT were no longer

significantly different from the controls, whereas eta and Cf decreased gradu

ally but remained higher than normal, BSR decreased, and the PFWD improved

by a factor of 3.8 times (D) and 3.3 times (ND). From these results, Li et al.

(1998) concluded that EGb has therapeutic effects in PAOD patients.

The effects of Ginkgo leaf concentrated oral liquor (GLC) on airway hyper

activity and inflammation, clinical symptoms and pulmonary functions of

asthma patients were determined in a randomized controlled trial (Li et al.,

1997). In contrast to placebo group, GLC significantly reduced airway hyperac

tivity ($P < 0.05$) and improved clinical symptoms ($P < 0.05$) and pulmonary Ginkgo Biloba

functions ($P < 0.05$) of the asthmatic patients. Thus, GLC is proposed as an

effective drug of anti-airway inflammation.

In addition to the numerous double-blind and randomized controlled trials

with Ginkgo biloba extracts dating back to 1984, six meta-analysis of clinical

studies with Ginkgo biloba have been reported (Table 6.5). These analyses

relate predominantly to diseases such as Alzheimer's, dementia, claudication,

cerebral performance and neurophysiology of patients with organic brain

syndrome where Ginkgo biloba has generally been found to have significant

positive clinical effects.

Recently, Ernst and Pittler (1999) systematically reviewed the clinical evi

dence of Ginkgo biloba preparations as a symptomatic treatment for dementia.

Computerized literature searches were performed to identify all double-blind,

randomized, placebo-controlled trials assessing clinical end points of Ginkgo

biloba extract as a treatment for dementia. Databases included Medline, Em

base, Biosis and the Cochrane Library. There were no restrictions regarding

the language of publication. Data were extracted in a standardized, predefined

fashion, independently by both authors. Nine double-blind, randomized, pla

cebo-controlled trials met the inclusion criteria and were reviewed. These

studies of varying methodological quality collectively suggest that Ginkgo

biloba extract is more effective for dementia than placebo. However, few,

generally mild, adverse effects were reported.

Flint and van Reekum (1998) reviewed the drug treatment of Alzheimer's

disease (AD) to provide guidelines for the physician on how to integrate these

treatments into the overall management of this disorder and made a qualitative

review of randomized, double-blind, placebo-controlled trials of medications

used to treat cognitive deficits, disease progression, agitation, psychosis or

depression in AD. A computerized search of Medline was used to identify

relevant literature published during the period of 1968-1998. Key words used

in the search were "randomized controlled trials" with "dementia" and with

"Alzheimer's disease." Donepezil and Ginkgo biloba, two of the four agents

currently available in Canada to treat the cognitive deficits of AD, were

associated with a statistically significant but clinically modest improvement

in cognitive function in a substantial minority of patients with mild to moderate

AD. These data indicate that selected medications can be used to treat cognitive

deficits, disease progression, agitation, psychosis and depression in AD. How

ever, considerable heterogeneity was observed in patients' responses to these

medications.

The effect of treatment with Ginkgo biloba extract on objective measures

of cognitive function in patients with AD was determined by Oken et al.

(1998) through a formal review of the current literature. According to these

authors, only four of the 50 studies examined met all inclusion criteria of

clear diagnoses of dementia and AD. In total, there were 212 subjects in each

of the placebo and Ginkgo treatment groups. Quantitative analysis of the TABLE 6.5. Meta-Analyses of Clinical Studies with Ginkgo biloba. Authors 1 Ernst and Pittler, 1999 Flint and van Reekum, 1998 Okenetal., 1998 Ernst, 1996 Hopfenmüller, 1994 Kleijnen and Knipschild, 1992 Type of Study Computerized literature searches for randomized controlled trials to March 1998 Literature review 1968-1998 of randomized double-blind placebo-controlled trials Review of 50 studies Review of 10 controlled trials Review of 11 controlled clinical trials Review of 40 trials Disease/Diagnosis Dementia Alzheimer's-cognitive deficits, agitation, psychosis or depression in AD Cognitive function in Alzheimer's disease Intermittent claudication Cerebrovascular insufficiency Cerebral insufficiency Assessment Nine studies out of 18 double-blind, randomized, placebo-controlled trials met the inclusion/exclusion criteria. Collectively, the trials suggest that Ginkgo biloba is efficacious in delaying the clinical deterioration of patients with dementia. Inconclusive data. GB associated with a significant but clinically

modest improvement in cognitive function. Small but significant effect of 3-6 months. Treatment with 120-240 mg of GB extract on cognitive function in AD. Studies implied that GB is an effective therapy for intermittent claudication. Seven studies confirmed effectiveness of GB vs. placebo based on total scores of clinical symptoms. Eight well-performed trials met the inclusion/exclusion criteria and confirmed the effectiveness of GB in cerebral insufficiency AD = Alzheimer's disease. GB = Ginkgo biloba extract.

literature showed a small but significant effect of three- to six-month treatments

with 120 to 240 mg ofGinkgo biloba extract on objective measures of cognitive

function in AD. The drug had no significant adverse effects in formal clinical

trials, but two cases reported bleeding complications (Oken et al., 1998),

Itil et al. (1998) compared the pharmacological effects of Ginkgo biloba

extract EGb with those of tacrine, also known as tetrahydroaminoacrine (THA),

one of the two currently approved drugs in the United States for the treatment

of Alzheimer's disease. The authors studied 18 subjects (11 males, seven

females of an average age of 67.4 years) with light to moderate dementia

[Mini Mental mean score = 23.7, ranges: 15-29 (Geriatric Depression Scale

mean scores = 3.7; range: 3.2-5.4)]. Each subject was randomly administered

a single oral "Test-Dose" of either 40 mg of tacrine or 240 mg of EGb

in two separate sessions within three- to seven-day

intervals. Before drug

administration and at one- and three-hour intervals after drug administration,

CEEGs were recorded for a minimum of 10 minutes. The CEEGs were ana

lyzed using Period Analysis. EGb induced pharmacological effects in the

central nervous system and exhibited more therapeutic effects (compared to

nonresponders) when drugs were administered chronically.

The effectiveness of Ginkgo biloba in the treatment of intermittent claudica

tion was evaluated by a systematic review of Medline-search-identified ten

controlled trials on the subject (Ernst, 1996). These were heterogeneous in

all respects and, with only few exceptions, of poor methodological quality. All

the studies implied that Ginkgo biloba is an effective therapy for intermittent

claudication. This hypothesis was confirmed in a monocenter, randomized,

placebo-controlled double-blind study with parallel-group comparison under

taken to demonstrate the efficacy of Ginkgo biloba special extract EGb 761

on objective and subjective parameters of the walking performance in trained

patients suffering from peripheral arterial occlusive disease in Fontaine stage

lib (Blume et al., 1996). A total of 60 patients were recruited (42 men; aged

47-82 years) with angiographically proven peripheral

arterial occlusive disease

of the lower extremities and an intermittent claudication existing for at least

six months. No improvement had been shown despite consistent walking

training, and a maximum pain-free walking distance on the treadmill of less

than 150 m was recorded at the beginning of the study. The therapeutic groups

were treated with either Ginkgo biloba extract EGb 761 at a dose of three

times one film-coated tablet of 40 mg per day by oral route or placebo over

a duration of 24 weeks following a two-week placebo run-in phase. The main

outcome measure was the difference of the walking distance between the start

of treatment and after 8, 16 and 24 weeks of treatment as measured on the

treadmill (walking speed 3 km/h and slope of 12%). As secondary parameters,

the corresponding differences for the maximum walking distance, the relative

increase of the pain-free walking distance, the Doppler index and the subjective

evaluation of the patients were analyzed. The absolute changes in the pain

free walking distance in treatment weeks 8, 16 and 24 as against the treatment

beginning (median values with 95% confidence interval) led to the following

values for the patients treated with Ginkgo biloba special extract EGb 761:19

m, 34 m and 41 m. The corresponding values in the placebo group were 7

m, 12 m and 8 m. The advantage of the EGb 761-treated group as compared

to the placebo group could be verified statistically at the three time points

with $p < 0.0001$, $p = 0.0003$ and $p < 0.0001$. The test for the presence of a

clinically relevant difference of 20% between EGb 761 and placebo also

produced a statistically significant result ($p = 0.008$). The Doppler index

remained unchanged in both therapeutic groups. A corresponding statistically

significant advantage for the EGb 761 group was observed on a descriptive

level for the other parameters tested. The tolerance of the treatment was very

good. The results of this placebo-controlled study show that treatment with

Ginkgo biloba extract EGb 761 produces a statistically highly significant and

clinically relevant improvement of the walking performance in trained patients

suffering from intermittent claudication with very good tolerance of the study

preparation.

Eleven controlled clinical trials were evaluated in a meta-analysis of studies

on the effectiveness of the Ginkgo biloba extract LI 1370 (Kaveri forte) in

patients with cerebrovascular insufficiency in old age (Hopfenmiiller, 1994).

All studies were placebo-controlled randomized double-blind trials, using in

most cases a daily dose of 150 mg extract. The requirements for the quality

of the studies were the basic criteria for the performance of clinical drug tests

analyzed from the biometrical scope. Three studies were excluded from the

meta-analysis according to methodological or objective reasons. In two further

studies, the evaluation of the physician or the patients was missing, therefore,

the studies could not be used for the analysis of the "global effectiveness."

All other studies were comparable with regard to diagnoses, inclusion and

exclusion criteria as well as methodology. Therefore, a statistical meta-analysis

could be performed for them, analyzing the parameters "single symptoms,"

total score of clinical symptoms and "global effectiveness." For all analyzed

single symptoms, significant differences could be concluded, indicating the

superiority of Ginkgo biloba in comparison to placebo. The analysis of the

total score of clinical symptoms from all relevant studies indicated that seven

studies confirmed the effectiveness (Ginkgo biloba being better compared to

placebo), while only one study was inconclusive (the medications were not

different).

Kleijnen and Knipschild (1992), by means of a critical review, sought

evidence from controlled trials in humans on the efficacy of Ginkgo biloba

extracts in cerebral insufficiency. The methodological quality of 40 trials on

Ginkgo and cerebral insufficiency was assessed using a list of predefined

criteria of good methodology, and the outcome of the trials was interpreted

in relation to their quality. A comparison of the quality was made with trials

of co-dergocrine, which is registered for the same indication. There were eight

well-performed trials out of a total of 40. Shortcomings were limited numbers

of patients included and incomplete description of randomization procedures,

patient characteristics, effect measurement and data presentation. In no trial

was double-blindness checked. Virtually all trials reported positive results. In

most trials, the dosage was 120 mg Ginkgo extract a day, given for at least

four to six weeks.

In addition to the studies described above, there is evidence of the use of

Ginkgo biloba preparations in combination with other phytochemicals for

treating patients suffering from viral diseases, especially AIDS (Beljanski,

1990). Apparently, the standardized hydrolysate of Ginkgo biloba extract

(Bioparyl) effectively mitigates the accumulation of gamma globulins pro

duced by HIV infection (Beljanski, 1990). Ginkgo biloba extract (EGb 761)

has also been found to decrease vasomotor disorder of the extremities during

a Himalayan mountain expedition (Roncin et al., 1996).

2.5. SAFETY

Th e seed and leaf of Ginkgo biloba, despite their medicinal properties, also

contain some toxic principles. Potent allergens such as ginkgolic acids are

present in the seed, pulp and leaves, and contact with the fresh seed pulp may

cause dermatiti s and other allergic reactions. Ginkgolic acid (125 jxg) causes

swelling of the lymph nodes when injected in mice (Van Beek et al., 1998).

Thus, the German Monograph E allows a concentration of less than 5 ppm

ginkgolic acid for the dry extract of Ginkgo biloba leaf. 4'-0-methylpyridoxine,

another potentially toxic compound present in edible Ginkgo nuts, may cause

convulsions an d loss of consciousness, particularly in children, when excessive

amounts of nuts are consumed. Food poisoning from the consumption of

Ginkgo biloba seeds has been attributed to the presence of the toxic substance

4'-6>-methylpyridoxine that antagonizes vitamin B6 and inhibits the formation

of 4-aminobutyric acid in the brain (Keiji et al., 1988).

Cytotoxicity due to

the antivitamin B6 from Ginkgo biloba seed has also been reported in 70

cases in Japan (Keiji et al., 1985). This toxin has also been detected in leaves

and some boiled Ginkgo-containing foods.

The crude alcoholic extract of the Ginkgo biloba leaf has been in use for

several years in France for the treatment of peripheral vascular disease, and

this has not been associated with any known side effects. Administration of

a single dose of the ginkgolide BN 52063 was not associated with any side

effects. However, since PAF seems to be involved in many natural processes,

such as the implantation of the human embryo in the uterus, and is present

in human amniotic fluid, it is worth remembering that these could be interfered

with. Toxicological studies in animals with the PAF antagonist BN 52021 do

not support such concerns (Chung and Barnes, 1988).

Guinot et al. (1988) studied the safety of BN 52063 in normal healthy

volunteers to find a maximum well tolerated dose for use in the initial and

multiple dose studies. Subsequent studies confirmed the PAF-antagonist activ

ity of BN 52063 in humans and investigated basic pharmacokinetics and

pharmacodynamics and a safe dose range. In a single-blind crossover trial,

five healthy male volunteers took increasing single doses of BN 52063, 20

mg, 40 mg, 80 mg, 120 mg and placebo, separated by at least one week. BN

52063 had no significant effects on blood pressure, pulse, electrocardiogram

and laboratory parameters at each dosage level. In a further two-week double

blind, randomized, crossover study, six healthy normal subjects were treated

with 40 mg BN 52063 or placebo, three times a day for a week, the two

treatment periods being separated by one week. Again, treatment with BN

52063 was found to be safe, well tolerated and significantly not different from

the placebo treatment. In a separate study, dosages of BN 52063 up to 720

mg as a single dose and 240 mg/day for two weeks or even 360 mg/day for

one week were well tolerated clinically and biologically by healthy volunteers

(Bonvoisin and Guinot, 1989).

2.6. CONCLUSIONS

More than 280 studies have been published on Ginkgo biloba since the

1950s, covering areas concerning the pharmacological and therapeutic effects

of Ginkgo biloba extract on the vascular tissue, impotence, memory dysfunc

tion and several other pathologies. At present, extracts of

Ginkgo leaves are

widely recommended in the Asian and European medical communities and

account for annual sales of approximately $500 million. In fact, during 1998,

physicians in Germany wrote more prescriptions (5.4 million) for Ginkgo

biloba extract than any other drug. It is also available in Europe and Asia as

an over-the-counter drug (OTC). The product is marketed in the U.S. and

Canada in dry powdered and liquid form, and in tablets and capsules for

oral use.

The very complex and unique structure of the macrocyclic lactones (gink

golides) impeded the study and function of these macromolecules for pharma

ceutical uses prior to 1988 (Braquet, 1988). Lactones similar to these structures

are now emerging as potentially important cancer chemotherapeutic agents

and are now in several human clinical trials (Wender et al., 1999). This

resurgence of interests in lactones will no doubt lead to revisiting the clinical

studies performed with ginkgolides in the late 1980s. This may spur new

studies that should be undertaken to reexamine the use of Ginkgo biloba for

cancer treatment in addition to traditional use in the treatment of various

diseases. The chemical, pharmacological and clinical

literature on the standard

ized extract of Ginkgo biloba is very impressive. However, although use of

acceptance in clinical studies in North America. Ginkgo biloba and its constit

uents also have proven applications in nonpharmaceutical, especially cosmetic,

industries (Van Beek et al., 1998). No doubt, these opportunities will be fully

explored as new studies on Ginkgo biloba and its constituents come to the

fore. At the dawn of the third millennium, the sixth for Ginkgo biloba, the

use of Ginkgo biloba appears to have no boundaries.

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