Pergamon

Food and Chemical Toxicology 36 (1998) 347–363

Food and Chemical Toxicology

Review of the Biological Properties and Toxicity of Bee Propolis (Propolis)

G. A. BURDOCK

Burdock and Associates, 622 Beachland Blvd, Suite B, Vero Beach, FL 32963, USA

(Accepted 23 August 1997)

Summary—Propolis is a multifunctional material used by bees in the construction and maintenance of their hives. Use of propolis by humans has a long history, predated only by the discovery of honey. Use of products containing propolis have resulted in extensive dermal contact and it is now increasingly being used a dietary supplement. Unlike many 'natural' remedies, there is a substantive database on the biological activity and toxicity of propolis indicating it may have many antibiotic, antifungal, antiviral and antitumour properties, among other attributes. Although reports of allergic reactions are not uncommon, propolis is relatively non-toxic, with a no-effect level (NOEL) in a 90-mouse study of 1400 mg/kg body weight/day

Abbreviations: ACF = aberrant crypt foci; AOM = azoxymethane; CAPE = caffeic acid phenethyl ester; CREF = cell line of rat embryo fibroblasts; DMAB = 3,2'-dimethyl-4-aminobiphenyl; DMSO = dimethyl sulfoxide; EEP = ethanol extract of propolis; GRAS = generally recognized as safe; HETE = hydroxyeicosatetraeroic acid; LOX = lipoxygenase; MC = methyl caffeate; NOEL = no-effect level; ODC = ornithine decarboxylase; PEC = phenylethyl caffeate; PED-MC = phenylethyl dimethylcaffeate; PEMC = phenylethyl-3-methylcaffeate; PI-PLC = phosphaticlylinositol-specific phospholipase C; PTK = protein tyrosine kinase; TPK = tyrosine protein kinase; WSD = water-soluble derivative.

Introduction

Identification and terminology

Propolis (CAS No. 9009-62-5) (sometimes also referred to 'bee glue') is the generic name for the resinous substance collected by honeybees from various plant sources (CHEMID, 1996). The word propolis is derived from the Greek *pro-,* for or in defence, and *polis*-, the city, that is, defence of the city (or the hive) (Ghisalberti, 1979). Propolis is a strongly adhesive, resinous substance collected, transformed and used by bees to seal holes in their honeycombs, smooth out the internal walls and protect the entrance against intruders. Honeybees (*Apis mellifera* L.) collect the resin from the cracks in the bark of trees and leaf buds. This resin is masticated, salivary enzymes added and the partially digested material is mixed with beeswax and used in the hive (Ghisalberti, 1979; Marcucci, 1995). Although propolis may contain some pollen, it is not pollen nor should it be confused with 'bee bread' or 'royal jelly', which are wholly different products of the hive.

The United States Department of Agriculture's 'United States Standards for Grades of Extracted Honey, Effective May 23, 1985' (adapted from 7 CFR §52.1394) describes propolis as follows (USDA, 1985):

> *(l)* **Propolis** *means a gum that is gathered by bees from various plants. It may vary in color from light yellow to dark brown. It may cause staining of the comb or frame and may be found in extracted honey.*

The precise composition of raw propolis varies with the source. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris (Cirasino *et al.*, 1987; Monti *et al.*, 1983). The wax and organic debris are removed during processing, creating propolis tincture.

The substance 'populus', a flavour ingredient, has been confused with propolis. This is an understandable misnomer since 'populus' is harvested from *Populus balsamifera* L. (and other *Populus* species), and it does refer to a resinous material in buds (i.e. before the leaves open in the spring). The buds are protected by a hood which contains a resinous,

0278-6915/98/$19.00 + 0.00
PII S0278-6915(97)00145-2

sticky, varnish-like substance*. This substance is extracted from the buds with a hydrocarbon solvent, producing an oleoresin which may be further steam-distilled or extracted with alcohol. The resulting extractant has a sweet, balsamic odour with a slight cinnamic undertone used in the flavouring of alcoholic beverages (Arctander, 1960; Burdock, 1995). Populus is chemically quite similar to propolis, since this resin is the raw material harvested by bees for manufacture of propolis (see below).

Historical and current uses of propolis

Man's long history of bee domestication has led to a thorough exploitation of bee products, and the many favourable properties of both raw and refined propolis lend to its application in many human pursuits. There is a long history of use of propolis, at least to 300 BC (Ghisalberti, 1979) and its use continues today in home remedies and personal products. Because propolis is reputed to have antiseptic, antimycotic, bacteriostatic, astringent, choleric, spasmolytic, anti-inflammatory, anaesthetic and antioxidant properties, the list of preparations and uses is nearly endless. These applications include over-the-counter dermatological items where it has been claimed useful in wound healing, tissue regeneration, treatment of burns, neurodermatitis, leg ulcers, psoriasis, morphoea, herpes simplex and genitalis, pruritus ani and activity against dermatophytes. It has been marketed as a treatment for rheumatism and sprains; and in dental medicine, it is claimed to be an anaesthetic five times as effective as cocaine. It is used in toothpaste and mouthwash preparations treating gingivitis, cheilitis and stomatitis. It has also found its way into pharmaceutical and cosmetic products such as face creams (vanishing creams and beauty creams), ointments, lotions and solutions. It is marketed in tablets, powder and chewing gum (Ayala *et al*., 1985; Bankova *et al*., 1983; Bjorkner, 1994; Dobrowolski *et al*., 1991; Esser, 1986; Ghisalberti, 1979; Hausen *et al*., 1987a; Marcucci, 1995). Although Europeans tend to use propolis-containing products more than Americans, it is sold in American health-food stores in capsules (approximately 50 mg/capsule) and is used in mass-marketed dental floss and toothpaste.

Antiquarian non-personal product or medicinal applications include propolis use in Italy in the 17th century, when Stradivari used propolis as an ingredient in the varnish of his stringed instruments (Monti *et al*., 1983). Today it is still used with musical instruments in rosin for stringed instruments and in the repair of accordions (Monti *et al*., 1983; Van Ketel and Bruynzeel, 1992). It has been proposed as a chemical preservative in meat products (Han and Park, 1995) and has been tested for bioactivity against larvae of the greater wax moth (*Galleria mellonella* L.), a common apiary pest, although little effect was noted (Johnson *et al*., 1994).

Current sales of propolis in the United States are estimated by the primary producer at 40,000 lb/yr (G.A. Burdock, personal communication, 1996). It is not possible to otherwise accurately estimate total sales of propolis in the US because bee-keeping and honey/propolis production is largely a cottage industry.

Propolis is consumed as a constituent of beeswax and honey. The 1975 monograph on beeswax by the Select Committee on GRAS Substances (SCOGS, 1975) notes the presence of 6% of beeswax as unidentified constituents, at least a portion of which is likely to be chrysine (1,3-dioxyflavone), a constituent of propolis. Bisson (1940) notes that the yellow colour of comb wax is due to the presence of chrysine and that propolis is a common constituent of the 'impurities' dissolved in beeswax.

Sources and processing of propolis

Botanical sources of propolis. Marcucci (1995) has noted that the compounds in propolis resin (raw, unprocessed propolis) originate from three sources: plant exudate collected by bees, secreted substances from bee metabolism, and materials which are introduced during propolis elaboration.

The source of the plant exudate was historically considered to be various indigenous poplar species, but this failed to explain why bees could produce propolis in the area of the equator where no poplars exist. Because the constituents of propolis reflect the source (see below, **Chemistry**) the advent of more sophisticated chemical analysis identified additional species of trees which could be used as a source of propolis for the foraging bees (Table 1).

Processing of propolis.. As noted above, propolis is a by-product of the beehive. Propolis is collected by the beekeepers who scrape the hive 'supers' (superstructure) with a hive tool. This usually takes place in the fall of the year after the honey is extracted. The propolis and wax mixture is shipped to the processor in boxes, barrels or bags.

Propolis involves little processing:

- The first step in processing is evaluation of the material on its arrival at the plant. If very waxy, it will be put through a cold-water washing process where the extrinsic wax will be removed. The remaining propolis is then air-dried on stainless-steel screens. If very little extrinsic wax is found, it will go immediately to the second step.
- The second step involves dissolving the propolis in 95% ethyl alcohol. Through a

*A possible contributing factor to this confusion may have been by Bisson (1940), who, in his treatise on beeswax, identified plant-elaborated 'bud-coating gums' as propolis.

Table 1. Botanical sources of propolis

Genus and species	Geographic location	Reference
Populus nigra, P. italica	Bulgaria	Bankova *et al*., 1983, 1994; Marcucci, 1995
Populus nigra	Albania	Bankova *et al*., 1994
Populus tremula	Bulgaria	Marcucci, 1995
Populus suaveolens	Mongolia	Bankova *et al*., 1994; Marcucci, 1995
Populus fremontii	USA (mainland)	Marcucci, 1995
Plumeria acuminata, Plumeria acutifolia	USA (Hawaiian islands)	Marcucci, 1995
Populus euramericana	United Kingdom	Marcucci, 1995
Betula, Populus, Pinus, Prunus and *Acacia* spp.; *Aesculus hypocastane*	Hungary	Marcucci, 1995
Betula, Alnus spp.	Poland	Marcucci, 1995
Delchampia spp.	Equatorial regions	Marcucci, 1995
Clusia spp.	Equatorial regions	Bankova *et al*., 1995; Marcucci, 1995
Clusia minor	Venezuela	Marcucci, 1995
Xanthorrhoea	Australia	Ghisalberti, 1979
Poplar, birch, elm, alder, beech, conifer and horsechestnut	"North temperate zone"	Ghisalberti, 1979

proprietary process, the remaining beeswax as well as bee parts and wood chips are removed.

- The final step involves filtration. The propolis tincture is put through a series of filters to remove any remaining small particles of foreign material.

Chemistry of propolis

Origin and composition of propolis. Propolis is a resinous, sticky gum, the colour of which varies from yellow–green to dark brown depending on its source and age. It can be likened to an aromatic glue. It is difficult to remove from the human skin, since it seems to interact strongly with the oils and proteins of the skin. It is hard and brittle when cold, but becomes soft and very sticky when warm (Ghisalberti, 1979; Koltay, 1981).

Some interesting points emerge from the limited work that has been carried out on the constituents of propolis. By far the largest group of compounds isolated are flavonoid pigments, which are ubiquitous in the plant kingdom. It is not surprising, therefore, that the same flavones have been isolated from different samples of propolis and the series of flavonoids isolated from propolis correlate reasonably well with those present in the plants from which honeybees collect propolis. It has been suggested that some of the flavones are modified by an enzyme in the honeybee. If so, it seems likely that any transformation must occur in the presence of enzymes in the saliva of the bees during collection. Also, the simple aromatic compounds found in propolis also occur commonly in plants and their presence in propolis is therefore not unexpected (Ghisalberti, 1979).

Johnson *et al*. (1994) assayed propolis from three geographical locations and produced the following results (Table 2). The gross composition of North American propolis (percent beeswax and methanol-soluble resin) is variable, but within the range of 16 to 80% reported for European propolis (Cirasino *et al*., 1987; Ghisalberti, 1979, Monti *et al*., 1983). The Johnson group note that the proportion of beeswax to plant resin is likely a compromise between availability and use. That is, propolis used to repair honeycomb is often supplemented with large quantities of wax to give it a firmer composition, while propolis applied in a thin coat to the surface of comb usually contains little or no wax (Meyer, 1956). Bees may also incorporate more wax into propolis during periods when resins are scarce or difficult to collect (Meyer, 1956). The low proportion of resin in propolis collected from south Georgia in this study may reflect a low availability of collectable resins in pine forests (Johnson *et al*., 1994).

Propolis collected from hives in Ohio was more chemically diverse (over 30 compounds detected by paper chromatography) than material from south Georgia (fewer than 10 major compounds) and contained a lower proportion of methanol-insoluble beeswax. Likewise, Rudzki and Grzywa (1983) found at least a slight difference in propolis gathered from the Warsaw region. However, the data of Johnson *et al*. (1994) revealed little variation in the chemical profile of specific hives over a 6-month period and no differences between propolis samples from adjacent hives.

Simple fractionation of propolis to obtain compounds is difficult due to its complex composition. The usual manner is to extract the fraction soluble in alcohol, called 'propolis balsam', leaving the alcohol-insoluble or wax fraction. Although ethanol extract of propolis (EEP) is the most common, extracts with other solvents have been carried out for identification of more than 200 constituents (Marcucci, 1995).

As noted earlier, the largest group of compounds isolated from propolis tincture is flavonoid pigments, which are ubiquitous in the plant kingdom and the series of flavonoids isolated from propolis correlate reasonably well with those present in the plants from which honeybees collect propolis. The substances identified in propolis are familiar constituents of food, food additives and/or generally recognized as safe (GRAS) substances.

Conspicuous among the list of constituents are hydroquinone (0.1%, Greenaway *et al*., 1987 and

Table 2. Percentage of extractable resin and insoluble residue (wax) in fractionated beeproplis collected from hives at three geographic locations (after Johnson *et al*., 1994)

	Ohio (N = 3)	North Georgia (N = 2)	South Georgia (N = 1)
Insoluble residue	25.1 ± 7.1a	55.3 ± 6.8b	76.0
Extractable resin			
Aqueous methanol	0.80 ± 0.7a	1.7 ± 0.4a	1.1
Petroleum ether I	0.93 ± 0.4a	4.4 ± 0.6a	0.1
Petroleum ether II	8.0 ± 12.9a	37.6 ± 4.8a	0.1
Ethyl acetate	62.7 ± 8.2a	14.4 ± 3.9b	22.0

Means with different letters are significantly different between Ohio and North Georgia sites.

1991) caffeic acid and its esters (2–20%, Bankova *et al*., 1995) and quercetin (< 0.1–0.7%, Greenaway *et al*., 1990), each of which have exhibited carcinogenic effects when administered to rodents. However, all three of these substances occur naturally in foods. Hydroquinone is present in beer and coffee (at levels of 1.25 to 40 ppm) and is approved as an indirect additive to food in §175.105; §§176.170, 180 and §177.2420. While quercetin and caffeic acids (and esters of caffeic acid) are not approved for use in food, the contribution of these substances through consumption of propolis is dwarfed when compared with consumption from other natural sources. For example, a single apple (with peel) may contain 5.8 to 26 mg quercetin (IARC, 1983). The estimated average daily intake quercetin by an individual in the US is 25 mg (NTP, 1992). Also, a single serving of lettuce* may contain 27–56 mg caffeic acid (IARC, 1993). Therefore, propolis contributes an insignificant amount of these substances when compared with the daily diet.

Possible contaminants of propolis. As with any natural product, contaminants from the environment are likely. However, analysis of several different lots confirm an absence of chlorinated hydrocarbons. Analytical data show the levels of lead in raw propolis at or below 9 ppb. Tinctures are generally below 5 ppb, but occasionally rise above this level.

Biological data

Biochemical aspects

Mechanism of action. As with any natural product, there are a number of constituents in propolis and its extracts that are held in common with other foods, some of which are also known to have biologic activity. Of those substances with biological activity, none contribute more to the observed effects of propolis than the flavonoids.

In his treatise on flavonoids, Havsteen (1983) divides the biochemical effects of flavonoids in animal systems into four categories: (1) binding affinity to biological polymers; (2) binding of heavy metal ions; (3) catalysis of electron transport; and (4) ability to scavenge free radicals.

Havsteen cites several examples of inhibition of a variety of enzymes by flavonoids including hydrolases and alkaline phosphatase. Propolis has exhibited similar effects inhibiting glycosyltransferases of cariogenic *Streptococci* (Ikeno *et al*., 1991), myeloperoxidase activity of inflammation (Frenkel *et al*., 1993), ornithine decarboxylase, lipooxygenase, tyrosine protein kinase and arachidonic acid metabolism (Rao *et al*., 1993). Further, it was shown by Coprean *et al*. (1986) and Gonzalez *et al*. (1995) that pretreatment of animals with propolis prior to administration of carbon tetrachloride (CCl_4) reduced the indices of pathological changes associated with CCl_4. A similar hepatoprotective effect was seen by Gonzalez *et al*. (1994) in paracetamol toxicity. Effects demonstrated by these latter studies reflect not only an inhibition of microsomal metabolism [also demonstrated in prolonged sleep times in hexobarbital-treated mice (Kleinrok *et al*., 1978)], but the additional antioxidative effects of propolis. Drogovoz *et al*. (1994) also cited antioxidative effects in rats 'with toxic liver damages of various duration and in acute hepatic ischemia.'

Although there are no definitive examples in the literature of propolis acting to bind heavy metal ions or acting to catalyse electron transport, the ability of flavonoids to suppress the formation of free radicals (Havsteen, 1983) may account for some of the antinflammatory effects seen with propolis. For example, the effectiveness of very small amounts of caffeic acid phenyl ester (CAPE) in ameliorating the inflammatory response induced by a tumour promoter (12-*o*-tetradecanoylphorbol-13-acetate), indicates that CAPE may be acting by interfering with the oxidative activation of the cells rather than by being antioxidants, which would require much greater amounts to scavenge the reactive oxygen species already produced (Frenkel *et al*., 1993).

Absorption, distribution and excretion. Although propolis contains a wide range of substances, the degree of absorption would probably not be different when these substances are consumed in any other food. The flavonoids exhibit a range of solubility and although they are consumed as glycosides

*Average serving 36 g Foods Commonly Eaten by Individuals: Amount Per Day and Per Eating Occasion (USDA Home Economics Research Report Number 44).

in their native state, bacterial glucosidases are capable of liberating the flavonoid for absorption (Havsteen, 1983). Further, orally administered flavonoids have been shown to appear in the urine (Havsteen, 1983).

Metabolism. Although definitive studies of the metabolism of propolis do not appear in the literature, the metabolism of many components of propolis is well known. The biologically most active fraction of propolis, the flavonoids, are known to be metabolized with no residues of flavonoids accumulating in the body (Havsteen, 1983).

Toxicity data

Acute toxicity studies. Acute toxicity tests of raw, unprocessed propolis would not be expected and because the method for propolis extraction remains unstandardized, variability in reported toxicity would be expected. None the less, a useable body of data exists. For example, Arvouet-Grand *et al*. (1993) reported the oral LD_{50} of propolis extract in the mouse to be greater than 7340 mg/kg, while Hrytsenko *et al*. (1977) reported an LD_{50} of 2050 mg/kg and an LD_{100} of 2750 mg/kg. Despite the disparity in the reported toxicities and the steepness of Hrytsenko's curve, there is nevertheless, a rather low innate toxicity for propolis extracts. Ghisalberti (1979) reported the works of Russian investigators who noted that an ether solution (extract?) of propolis was not toxic to white mice at doses of 350 mg/kg and that the LD_{50} after 19 hr for both ether and alcohol extracts was 700 mg/kg. Ghisalberti (1979) also reported that cats tolerated subcutaneous administration of 100 mg/kg of an ether extract of propolis.

Dobrowolski *et al*. (1991) administered approximately 700 mg/kg orally to groups of 10 mice (five male and five female) and monitored them up to 48 hr post-dose. They reported that the propolis preparations were well tolerated and that no deaths occurred during the 48-hr observation period.

If one considers the flavonoids to be the primary biologically active constituent of propolis extracts, this relatively low toxicity of the extracts is predictable, since flavonoids are themselves of rather low toxicity. For example, the flavonoid pinocembrin, the predominant flavonoid in several extracts, showed no toxicity when administered orally to mice at 1000 mg/kg (Metzner *et al*., 1977). Further, Havsteen (1983), in his review of flavonoids, reports the LD_{50} of flavonoids to be from 2000 to 10,000 mg/animal (8000 to 40,000 mg/kg for a 250-g rat).

Kleinrok *et al*. (1978) performed a pharmacology screen on an ethanolic extract of propolis (EEP) on male BalbC mice and male rats of the Wistar strain. All administration was intraperitoneal. They recorded a dose-responsive decrease in spontaneous movement of mice at from 100 to 2000 mg/kg; subthreshold doses for this parameter were 1–400 mg/kg in rats and 10–100 mg/kg in mice. EEP in doses of 50–400 mg/kg intensified the hypothermic effect of dimethyl sulfoxide (DMSO). The authors state that EEP had 'an effect on the narcotic action of chloral-hydrate,' which they contrast to an EEP produced prolongation in hexobarbital sleeping time. Further, they note that EEP does not have an effect on the DMSO decrease in amphetamine-hyperactivity in mice and that there was 'no substantial effect' on respiration rate of animals given 'narcotics.' The authors conclude that EEP had a 'weak general effect' on the animals.

Irritancy testing was performed in guinea pigs by open epicutaneous application of three different dilutions of propolis (20%, 10%, 1% in acetone) onto the clipped and shaved flank and read after 24 hr. The threshold of irritation for propolis was found to be higher than the 20% solution (Hausen *et al*., 1987b). Arvouet-Grand *et al*. (1993) reported that application of both propolis extract alone and in ointments to rabbits was not irritating. Neither of these findings are surprising in a consideration of the widespread use of propolis in cosmetics and skin creams.

Multiple dose toxicity studies. DeCastro and Higashi (1995) experimented with the anti-trypanosomal effects of propolis via several different routes. They reported that daily oral administration of propolis (in ethanol) from 200 to 1220 mg propolis/kg/day for 7–10 days did not alter the progress of the parasitemia or change the mortality of propolis-treated *v*. non-treated mice. Similar results were seen in animals treated with 1600 or 4000 mg/kg/day in drinking water and in groups administered propolis in the diet at a rate of 2500 and 5000 mg propolis/kg/day (the number of days of administration was not clearly stated).

Suspensions prepared from ethanolic extracts of Brazilian and Chinese propolis were fed to 5-wk-old mice at doses of 2230 to 4000 mg/kg. After 2 wk of treatment, no deaths were noted, body weights had increased normally and no abnormalities were found on necropsy (Kaneeda and Nishina, 1994).

Hollands *et al*. (1991) undertook a series of experiments using an alcoholic extract of propolis administered in the drinking water to both rats and mice. Controls received alcohol in water and or water alone. In the first two experiments, Wistar rats received 1875 mg/kg/day propolis in the drinking water for 30 days, or 2470 mg/kg/day propolis in the drinking water for 60 days. In a comparison to each respective control, there were no changes in the clinical appearance, behaviour, urine output, body weight or mortality. No histological changes were seen in the 30-day study and the only change seen in the 60-day study was minor hepatic necrosis in one animal in the alcohol control group. The remaining changes were not treatment related.

In the first of three experiments in mice, Hollands *et al*. (1991) treated mice with propolis in

the drinking water to produce a consumption of approximately 4600 mg propolis/kg/day for 90 days. Blood samples were taken from four animals in each of the propolis-treated, alcohol vehicle-treated and water controls for examination of 'blood glycemia', cholesterol and urea. As in the rat study, there were no differences in clinical appearance, behaviour, urine output, body weight or mortality; nor was there a difference in 'glycemia' or cholesterol. There was an increase in mean urea values in both the propolis group and in the alcohol group at ×1.5 and ×3.3 water control values, respectively. Two follow-up experiments were conducted in mice, with the animals administered 6 mg propolis/ml drinking water, which if water consumption is consistent with the preceding experiment, the animals received 1400 mg/kg/day for 14 and 90 days, respectively. At this lower dose, there was no difference in blood urea between any of the groups. No histomorphological changes were seen in any of the mouse studies. The authors attribute the increase in blood urea at the high dose to be an effect of the alcohol, not the propolis. This dose level of 1400 mg/kg/day in mice is proposed as a NOEL (no-effect level).

In an experiment to determine the effects of an alcoholic extract of propolis on carcinogenesis in rats, Ikeno *et al.* (1991) administered propolis at a rate of 1 mg/ml in drinking water for 63 days. No animal deaths or toxicity resulting from the propolis were reported and body weights were comparable to control. There were no differences between control and propolis-treated animals in serum glucose or amylase activity in serum, pancreas, parotid gland or liver. If it can be assumed that rats consume an amount of water per day equivalent of 10% of their body weight, a 150-g rat in this experiment would have consumed 150 mg/day or 1000 mg/kg day.

Antitumour effects. The antitumoral effect of EEP was demonstrated in mature mice bearing Ehrlich carcinoma. Survival rate after EEP treatment (0.5 ml 0.25% EEP) was compared with that of bleomycin (0.001%), each given alone or in combination every 2 days for 36 days and followed up for 14 additional days. The survival rate of the mice at 50 days was 55% after EEP and 40% after bleomycin, while all the mice treated with the EEP + bleomycin combination demonstrated shorter survival than the controls. The authors concluded that the antitumour effect of propolis was due to the flavonoids inhibiting the incorporation of thymidine, uridine and leucine into the carcinoma cells, thus leading to an inhibition of DNA synthesis. The reduced activity of bleomycin and EEP administered simultaneously is attributed to reduced activity of bleomycin in the presence of EEP-containing cytochrome C reductase inhibitors (Scheller *et al.*, 1989).

More recently, work on the antitumoral/anti-inflammatory activity of propolis has concentrated on caffeic acid phenyl esters. Frenkel *et al.* (1993), noting that CAPE is cytotoxic to tumour and virally transformed cells, but not to normal cells (see also below, ***In vitro* testing**). These investigators wanted to establish whether CAPE inhibits the tumour promoter (12-*o*-tetradecanoylphorbol-13-acetate)-mediated oxidative processes that are considered characteristic for tumour promotion, for example TPA-induced ear oedema and ornithine decarboxylase (ODC) inhibition, polymorphonuclear leucocyte infiltration, hydrogen peroxide formation and formation of oxidized bases in epidermal DNA. They found clear inhibition by CAPE of TPA-induced ear oedema and epidermal ODC in CD-1 and SENCAR mice. In CD-1 mice, CAPE decreased TPA-induced oedema and ODC and was about twofold more potent than its parent, caffeic acid. SENCAR mice were more sensitive than CD-1 mice to the action of CAPE with respect to oedema, but not ODC induction. CAPE was also found to decrease TPA-induced PMN infiltration, as indicated by decreased myeloperoxidase activity. CAPE inhibited (by 86%) TPA-induced hydrogen peroxide formation and inhibited the formation of oxidized bases, as measured by 5-hydroxymethyluracil and 8-hydroxylguanine. As can be seen from (Table 3), the reduction in effects of TPA is not always greater at higher doses and, the authors suggest that at high doses there may be some interference with intracellular processes. However, the effectiveness of these very small amounts indicates that CAPE may be acting by interfering with the oxidative activation of the cells rather than by being antioxidants, which would require much greater amounts to scavenge the reactive oxygen species already produced. (Frenkel *et al.*, 1993).

Rao *et al.* (1993) investigated the effect of propolis constituents for antitumour activity against colon carcinogenesis. The first study was designed

Table 3. CAPE-mediated effects of TPA-induced inflammatory changes in the skin of SENCAR mice

Treatment	MPO units/cm^2 skin	H_2O_2 nmol/cm^2 skin	HmdUrd/10^4 bases	8-OHdGua/10^4 bases
Acetone	0.1 ± 0.03	12.8 ± 0.8	16.2 ± 7.7	5.0 ± 2.0
TPA (6.5 nmol)	23.7 ± 7.6	31.5 ± 0.7	39.4 ± 6.4	11.8 ± 6.3
TPA/CAPE (6.5 nmol)	5.9 ± 0.3	15.7 ± 2.2	13.1 ± 0.6	1.4 ± 0.8
TPA/CAPE (650 nmol)	10.8 ± 2.0	11.6 ± 0.6	30.5 ± 10.5	9.4 ± 1.1

Mice were preincubated with CAPE at the designated dose, then treated with 6.5 nmol TPA. Results are expressed as mean values of two to five experiments ± SE.

to investigate several of the inhibitor effects of methyl caffeate (MC) and phenylethyl caffeate (PEC) on azoxymethane (AOM)-induced ODC, tyrosine protein kinase (TPK) and arachidonic acid metabolism in liver and colonic mucosa of male F344 rats.

In this study with MC and PEC, rats were started, at 5 wk of age, on a diet of 600 ppm of either MC or PEC. At 7 wk of age, test animals were injected subcutaneously with AOM once weekly for 2 wk. The animals were killed 5 days later and colonic mucosa and liver were analysed for ODC, TPK, lipoxygenase and cyclooxygenase metabolites. The investigators found the PEC diet significantly inhibited AOM-induced ODC and TPK activities in the liver and colon. The PEC diet also significantly suppressed the AOM-induced lipoxygenase metabolites 8(S)- and 12(S)-hydroxyeicosatetraenoic acid (HETE). The animals fed the MC diet exhibited a moderate inhibitory effect on ODC and 5(S)-, 8(S)-, 12(S)- and 15(S)-HETEs and a significant effect on colonic TPK activity. However, the MC and PEC diets showed no significant inhibitory effects on cyclooxygenase metabolism. In a follow-up *in vitro* study, caffeic acid and MC showed inhibitory effects on HETE formation only at a 100 mM concentration, whereas PEC, phenylethyl-3-methylcaffeate (PEMC) and phenylethy dimethylcaffeate (PEDMC) suppressed *in vitro* HETE formation in a dose-dependent manner (Rao *et al*., 1993). These investigators also reported the effects of PEC, PEMC or PEDMC on AOM-induced aberrant crypt foci (ACF) formation in the colon of F344 rats. In this experiment, the 5-wk-old rats were fed the caffeate esters at 500 ppm and were treated from wk 7–9 with AOM (subcutaneously, once weekly for 2 wk at a dose of 15 mg/kg). These animals were kept on diet until 16 wk of age, then killed. The results showed that PEC, PEMC and PEDMC inhibit AOM-induced ACF formation to 55.2%, 82.1% and 81.3%, respectively. The authors note that these results are significant since ACF represent the precursor lesions of chemically-induced colon cancer (Rao *et al*., 1993). Therefore, inhibition of such lesions is strongly suggestive of a protective effect of these esters against colon cancer.

In a follow-up study, Rao *et al*. (1995), expanded the investigation on the effect of PEMC, the most active inhibitor of ACF-induced lesions in the rat colon. In this study, the objective was to examine the chemopreventive action of dietary PEMC on azoxymethane-induced colon carcinogenesis and the modulating effect of PEMC on phosphatidylinositol-specific phospholipase C (PI-PLC), phospholipase A2, lipoxygenase (LOX) and cyclooxygenase activities in the colonic mucosa and tumor tissues in male F344 rats.

At 5 wk of age, groups of rats were fed the control diet or a diet containing 750 ppm PEMC. At 7 wk of age, all animals except those in the vehicle (normal saline)-treated groups were given 2-weekly subcutaneous injections of azoxymethane at a dose rate of 15 mg/kg body weight/week. All groups were maintained on their respective dietary regimen until the termination of the experiment 52 wk after the carcinogen treatment. Colonic tumours were evaluated histopathologically. Both colonic mucosa and tumours were analysed for PI-PLC, phospholipase A2, cyclooxygenase and LOX activities.

In their general observations at the conclusion of the study, the authors noted that the body weights of animals treated with vehicle or AOM and fed the control or PEMC diets were similar throughout the study. In vehicle-treated animals, the feeding of PEMC did not produce any gross changes in liver, kidney, stomach, intestine or lungs, nor any kind of histopathological changes in the liver or intestine attributable to toxicity.

The investigators found that dietary PEMC significantly inhibited the incidence and multiplicity of invasive, non-invasive, and total (invasive plus non-invasive) adenocarcinomas of the colon ($P < 0.05$–0.004), intestine (total of small intestine and colon) and ear-duct tumours. Dietary PEMC also suppressed the colon tumour volume by 43% compared with the control diet.

Animals fed the PEMC diet showed significantly decreased activities of colonic mucosal and tumour PI-PLC (about 50%), but PEMC diet had no effect on phospholipase A2. The product of 5(S)-, 8(S), 12(S)- and 15(S)-HETEs via the LOX pathway from arachidonic acid was reduced in colonic mucosa and tumours (30–60%) of animals fed the PEMC diet as compared with control diet. PEMC had no effect on the formation of colonic mucosal cyclooxygenase metabolites, but inhibited the formation in colonic tumours by 15–30%. The precise mechanism by which PEMC inhibits colon tumorigenesis remains to be elucidated. It is likely that the chemopreventive action may be related, at least in part, to the modulation of PI-PLC-dependent signal transduction and LOX-mediated arachidonic acid metabolism (Rao *et al*., 1995).

The sum of these studies is the provision of compelling evidence of the anti-inflammatory and anticarcinogenic properties of propolis extracts or derivatives when tested in animal models. These observations correlate well with the *in vitro* studies described below, but first, an understanding of the cytotoxic nature of propolis is required.

Cytotoxicity

Common pathogens and higher plants: In its native application, a primary function of propolis in the hive is to act as a biocide, and may act against invasive bacteria, fungi and even invading larvae (Ghisalberti, 1979; Lisowski, 1984; Marcucci, 1995).

Propolis may also function in the embalming of intruders. For example, Koltay (1981) discovered the propolis-entombed corpse of a mouse which had been well preserved for nearly a year.

There are a number of studies documenting the biocidal functions of propolis, its extracts and constituents (Table 4). As can be seen in the table, the activity is fairly broad spectrum with activities against $Gram^+$ and $Gram^-$ rods and cocci, yeasts and fungi, among them organisms associated with varying degrees of pathogenicity in man and other animals, including human tuberculosis bacilli. Extracts have been shown to inhibit the elaboration of toxins [e.g. ochratoxin A by *Aspergillus sulphureus* (Pepeljnjak *et al*., 1982)] and formation of water insoluble-glucans required by cariogenic *Streptococci* to adhere to tooth enamel (Ikeno *et al*., 1991).

Although the reported degree and scope of activity among the general categories of susceptible organisms is variable it is, in a sense, markedly similar, with activities generally below 10 mg/ml. The difference might be attributed to a difference in virulence of the test organisms and to a difference in the flavanoids content. For example, Pepeljnjak *et al*. (1985) correlated the flavonoid content with activity against *Bacillus subtilis* and that flavonoid content varied considerably with the 38 samples gathered in parts of Croatia with differing climate and vegetation. Also, as would be expected, the method of extraction produced variability of results as noted by Spiridonov *et al*. (1992) comparing propolis extracts made with water or 40% or 96% ethanol. The efficacy of the flavanoids is succinctly demonstrated by the work of Metzner *et al*. (1977), with a difference in efficacy between propolis and a constituent flavonoid (i.e. pinocembrin) somewhere between one- and 10-fold.

Interestingly, growth, germination and/or mitosis was reported inhibited in *Vicia faba, Hordeum vulgare, Allium cepa* and *Allium sativum* (Abdou and Omar, 1988). This supports a report that a potato placed inside a hive was sealed with propolis and failed to sprout; similar phytoinhibitory properties have been described for lettuce seedlings, rice grains and seeds of *Cannabis sativa* (Ghisalberti, 1979). This probably represents an important survival mechanism by preventing the sprouting or invasion of plant life into the hive.

Conspicuous among failures to control pathogens by propolis, is the non-efficacious or marginal activity against the parasites *Entamoeba histolytica, Toxoplasma gondii, Trichomonas vaginalis* or *Trypanosoma cruzi, in situ*. For example, DeCastro and Higashi (1995) administered up 5000 mg/kg/day to mice without significant interference to the moribund progress of the *T. cruzi* infestation, but high doses of conspicuously more toxic therapeutic agents are often required for control of this organism (Webster, 1990). *Entamoeba histolytica, Toxoplasma gondii* and *Trichomonas vaginalis* were incubated *in vitro* with various concentrations of propolis or propolis extracts with no activity against *E. histolytica*. Activity against *Toxoplasma gondii* and *Trichomonas vaginalis* was evident only after 24 hr of incubation with propolis extracts at concentrations of 150 mg/ml (Dobrowolski *et al*., 1991; Starzyk *et al*., 1977).

Viruses and transformed cells: In addition to other biocidal properties, propolis and its extracts clearly have viricidal properties as well. Amoros *et al*. (1992) investigated the *in vitro* effect of propolis on several DNA and RNA viruses including herpes simplex type 1 (an acyclovir resistant mutant), herpes simplex type 2, adenovirus type 2, vesicular stomatitis virus and poliovirus type 2. The inhibition of poliovirus propagation was clearly observed through a plaque reduction test and a multistep virus replication assay with a selectivity index equal to 5. At the concentration of 30 μg/ml, propolis reduced the titre of herpes viruses by 1000, whereas vesicular stomatitis virus and adenovirus were less susceptible. In addition to its effect on virus multiplication, propolis was also found to exert a virucidal action on the enveloped viruses HSV and VSV (Amoros *et al*., 1992).

Maksimova-Todorova *et al*. (1985) reported that various fractions of propolis effected the replication of influenza viruses A and B, vaccinia virus and Newcastle disease virus.

Substances isolated from propolis have also been examined for antiviral activity. Serkedjieva *et al*. (1992) showed that isopentyl ferulate inhibited the infectious activity of influenza virus A/Hong Kong (H_3N_2). Debiaggi *et al*. (1990) examined several different flavonoids of propolis (acacetin, kaempferol, chrysine, quercetin and galangin), using a battery of viruses. Two of the flavonoids studied, chrysine and kaempferol, were highly active in inhibiting the replications of several herpes viruses, adenoviruses and a rotavirus. The flavonoids acacetin and galangin were not active in the viruses studied even at concentrations 100 times greater than chrysine and kaempferol. Quercetin was least effective of all.

A 5% alcoholic propolis solution given intranasally or as an aerosol 2 hr before infection completely inhibited influenza virus proliferation in mice. The preparation had no effect when given to mice already infected. It had no toxic effects, at this dose, to mice or other experimental animals (Schevchenko *et al*., 1972).

In vitro studies. Hladon *et al*. (1980) noted citations in the literature about the successful uses of propolis as a bacteriostat and fungistat. They followed with a report of the successful suppression of growth of HeLa cell and human nasopharynx carcinoma cells (KB cells) with ether and butyl alcohol extractions of propolis (Table 5). This work was shortly followed by that of Ban *et al*. (1983) who,

Table 4. Cytotoxic activity of propolis and its extracts

Substance	Test organism	Comments/results	Reference
Propolis	*Candida, Saccharomyces, Cryptococcus*	Minimum inhibiting concentrations of 3–10 mg/ml	Metzner *et al.*, 1977
Pinocembrin (5,7-dihydroxyflavone)	*Candida, Saccharomyces, Cryptococcus*	Minimum inhibiting concentrations of 0.1–3 mg/ml	Metzner *et al.*, 1977
Propolis (ethanolic extract)	Several genera, including *Mycobacteria, Saccharomyces, Candida*	Ethanolic extract of propolis was found to have antibacterial activity against a range of commonly encountered cocci and $Gram^-$ and $Gram^+$ rods, including human tuberculosis bacilli. In screening studies, different concentrations of ethanolic extract of proplis completely inhibited the growth of *Bacillius megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Bacillus brevis, Staphylococcus aureus, Listeria monocytotenes, Pseudomonas aeruginosa* DSM 50071, *Klebsiella pneumoniae, Streptococcus* spp. *Enterobacter aerogenes, Saccharomyces cerevisiae*, and *Candida albicans*, but it did not inhibit the growth of *E. coli* ATCC 25922.	Digrak *et al.*, 1995
Propolis (ethanolic extract)	*Asperigillus sulphureus* NRRL 4007	Concentrations of propolis were 0.25, 0.50, 1.0 and 2.0 mg/ml. Growth of the microorganism was inhibited at all concentrations up to day 10 of incubation, only the 2.0 mg/ml concn of propolis exhibited fungistatic activity. The amounts of ochratoxin A were proportional to the growth of *A. sulphureus* and reciprocal to the amounts of propolis extract used.	Pepeljnjak *et al.*, 1982
Propolis	*Crytococcus neoformans, Histoplasma encapsulatum, Madurella mycetomi, Microsporum canis, Microsporum gypseum, Phialophora jeanselmei, Piedra hortae, Trichophyton mentagrophytes, Trichophyton rubrum, Trichosporon cutaneum*	Propolis granules as well as prepared propolis tablets were used. Incubations were carried out at concentrations of from 5 to 25 mg/ml. Antifungal activity was noted with one or the other preparation against *Microsporum canis, Microsporum gypseum, Phialophora jeanselmei, Piedra hortae, Trichophyton mentagrophytes, Trichophyton rubrum* and *Trichosporon cutaneum*. Griseofulvin exhibited a broader spectrum and a potency of approximately 100 times greater than either of the propolis preparations.	Dobrowolski *et al.*, 1991
Propolis	*Bacillus subtilis* (IP-5832	Thirty-eight propolis samples were collected in several regions of SR Croatia differing in climate and vegetation. Amounts of 3,5,7-trihydroxyflavone and 5,7-dihydroxyflavone were determined and concentrations were correlated with the growth inhibition.	Pepeljnjak *et al.*, 1985
Propolis	Gram positive organisms: *S. aureus, S. pyogenes, S. viridans, D. pneumoniae*, and *C. diphtheria*. Gram negative organisms: *E. coli, S. typhi, S. paratyphi-A, S. paratyphi-B* and *S. flexneri*	Propolis exhibited some (marginal) antibacterial activity, particularly against Gram-positive organisms.	Dobrowolski *et al.*, 1991
Propolis (ethanol extract)	*Streptococcus sobrinus* 6715, *S. mutans* and *S. crcetus*	Antimicrobial action exhibited *in vitro* as zone of inhibition and *in vivo* as fewer caries in animals receiving propolis in drinking water.	Ikeno *et al.*, 1991
Propolis (alcoholic extract)	*Staphylococcus aureus* 209	Antibacterial activity associated with phenolic acid fraction. The authors comment that no antibacterial activity in the batch of propolis with a low phenol content.	Bankova *et al.*, 1995
Propolis (alcoholic extract)	*Mycobacterium* (30 clinical strains	The inhibitory and bactericidal minimum concentrations (IMC and BMC), respectively) were determined for 1 and 7 days of treatment. All *Mycobacterium* strains with the exception of *M. tuberculosis* were inhibited with 1.0 mg/ml propolis. Only 30% of the *M. tuberculosis* strains tested were inhibited by 2.0–5.0 mg/ml propolis. The difference in BMC for 1 and 7 days of treatment was not significant.	Rojas Hernandez *et al.*, 1993
Propolis	*Entamoeba histolytica*	No activity	Dobrowolski *et al.*, 1991
Propolis (ethanol extract)	*Toxoplasma gondii*	Organism mixed with propolis extract (150 mg?/ml) and incubated. All organisms dead following a minimum of 24 hr incubation.	Starzyk *et al.*, 1977
Propolis (alcoholic extract)	*Trypanosoma cruzi*	Oral administration of up to 1.2 g propolis extract/kg per day or propolis offered *ad lib.* in the drinking water (up to 4 g/kg/day) or added to the food (up to 5 g/kg/day) did not interfere with parasitemia kinetics or survival rate of *Trypanosoma cruzi*-infected mice.	DeCastro and Higashi, 1995
Propolis (alcoholic extract)	*Trichomonas vaginalis* (3 strains	Lethal activity on all three strains at 150 mg/ml.	Scheller *et al.*, 1977
Propolis (ethanol extract)	*Trichomonas vaginalis* (3 strains	Lethal within 24 hr at 150 mg/ml (*in vitro*); survival extended at lower concentrations.	Starzyk *et al.*, 1977
Propolis	*Vicia faba, Hordeum vulgare, Allium cepa* and *Allium sativum*	Root tip cells were treated with propolis in distilled water for 24 and 48 hr at 0.0625, 0.25 or 1%. Mitosis was inhibited, the effect increasing with increase in concentration and exposure time. Chromosome aberrations such as tetraploid cells, C-metaphase and mitodepression were induced, signifying effects on spindle function. Germination of *V. faba* and barley was adversely affected.	Abdou and Omar, 1988

using the more commonly reported alcoholic extract of propolis, demonstrated an inhibitory concentration (IC_{50}) of 10 μg extract/ml against HeLa cells. Similar work has been reported by others, including Spiridonov *et al.* (1992), who experimented with water and ethanolic extracts of propolis.

Grundberger *et al.* (1988) identified caffeic acid as one of a class of constituents responsible for much of the antibiotic and antiinflammatory properties of propolis. Using an Ltk^- growth inhibition assay, they identified CAPE as one biologically active substance that lent itself to easy synthesis in large quantities.

Results of Grundberger *et al.* (1988) in various cell lines is presented in Table 5. They noted that mouse cells (C3H $10T\frac{1}{2}$ and Ltk^-) were most sensitive, with concentrations as low as 2.5 μg/ml CAPE effectively blocking the increase in number of $10T\frac{1}{2}$ cells. Interestingly, benzo[*a*]pyrene-transformed $10T\frac{1}{2}$ cells exhibited increased resistance to CAPE action requiring up to 20 μg/ml CAPE for 80% inhibition. In contrast, normal rat 6 cells were less sensitive to CAPE than those transformed by T24 oncogene. The growth of two monkey cell lines, CV1 and Vero, suffered severe inhibition only at concentrations of CAPE greater than 10 μg/ml.

These investigators (Grundberger *et al.*, 1988) explored the differential effect on normal and transformed cells with a cell line of Fischer rat embryo fibroblasts (CREF) and its counterpart, transformed by adenovirus serotype 5 (wt3A). After 72 hr and at CAPE concentrations as high as 8 μg/ml, approximately 75% of the CREF cells remained unaffected, yet under the same conditions, the wt3A cells were nearly 90% inhibited. Similar effects were observed after 24 and 48 hr treatments. Although the authors did not speculate on a specific mechanism for this difference, they did find that ^{3}H-thymidine incorporation was inhibited in human breast carcinoma (MCF-7) and melanoma cell lines (SKMEL-28 and SK-MEL-170) in culture. Similar inhibitions were observed for HT29 colon and renal carcinoma lines. They concluded that human tumour cell lines displayed a significantly greater sensitivity to the action of CAPE than analogous normal lines. Similar results were reported by Su *et al.* (1991 and 1994) using CREF and (adenovirus) Ad5-transformed CREF cells and importantly, that possession of the transformation genotype is not enough to undergo growth suppression, but only when the transformed phenotype is expressed (Su *et al.*, 1994).

Similar growth suppression was demonstrated by Rao *et al.* (1992) using MC, PEC and PEDMC. These investigators also reported decreased levels of ODC and protein tyrosine kinase (PTK), both indications of transformation.

Matsuno (1995) isolated a clerodane diterpenoid from propolis and reported growth of human hepatocellular carcinoma cells (HuH 13) inhibited at 10 μg/ml and lethality at 20 μg/ml, lethality to human lung carcinoma (HLC-2) at 50 μg/ml, but importantly, greater than 75% survival of (normal) human diploid foreskin and primary rabbit kidney cells at 100 μg/ml. Matsuno (1995) also reported cytotoxicity to HeLa, KB and rat W3Y cells.

Using a variety of methods, Chiao *et al.* (1995) showed that cell death induced by CAPE in the transformed Wt3A cells was apoptosis. Under the same CAPE treatment conditions, CREF cells transiently growth arrested. Using a variety of agents and manipulations, the authors concluded that CAPE can modulate the redox state of the cells. Sensitivity of the cells to CAPE-induced cell death may be determined by the loss of normal redox state regulation in transformed cells. (Chiao *et al.*, 1995).

Mutagenicity studies. Because some of the observed biological activities of propolis may be due to caffeic acid (cinnamic acid) esters present in the propolis, Rao *et al.* (1992) investigated the antimutagenic effect of these esters. The authors synthesized three caffeic acid esters, MC, PEC and PEDMC and tested them against the 3,2'-dimethyl-4-aminobiphenyl (DMAB, a colon and mammary carcinogen)-induced mutagenicity in *Salmonella typhimurium* strains TA98 and TA100. Neither the parent compound, caffeic acid, nor any of the esters were mutagenic with or without S-9 activation. As expected, DMAB at 5 and 10 μg was mutagenic in both TA98 and TA100. When DMAB was tested in the presence of the esters, DMAB-induced mutagenicity was significantly inhibited with 150 μM MC, 40–60 μM PEC and 40–80 μM PEDMC in both tester systems. (Rao *et al.*, 1992).

Immune response studies. The list of applications of propolis and its extracts is nearly endless and many are described above. As a result of this wide utilization of propolis, reports of allergic reactions have been identified for nearly all occupations and all parts of the body. For example, in their excellent set of reviews of propolis allergy, Hausen *et al.* (1987a), report affected occupations to include beekeepers, artists, housewives, honey extractors, a tailor, a physician and an engineer. Reported affected parts of the body include, but are not limited to, the hands, forearms, face, neck, perioral region, feet, eyelids, external ear, vulva and penis. There are also reports of pets affected as the result of owners using propolis-containing home remedies (Hausen *et al.*, 1987a). Bjorkner (1994) reports that some of these dermatites may also result from airborne contamination.

Although DeGroot *et al.* (1994) report that poplar bud constituents are probably responsible for allergy to propolis, Valsecchi and Cainelli (1984) reported subjects who, although responsive to patch testing with propolis, beeswax and balsam

Table 5. *In vitro* studies

Substance	Test medium	Comments/results	Reference
Propolis (alcoholic extract)	HeLa cells	$IC_{50} = 10$ μg extract/ml	Ban *et al.*, 1983
Propolis (diethyl ether extract)	HeLa cells	$ED_{50} = 3.9$ μg extract/ml of diethyl ether extract most effective	Hladon *et al.*, 1980
Propolis (diethyl ether extract, followed by butyl alcohol extraction)	HeLa cells; Human KB cells (nasopharynx carcinoma	HeLa cells $ED_{50} = 2.6$ μg/ml extract. Human KB cells $ED_{50} = 2.9$ μg/ml extract	Hladon *et al.*, 1980
Propolis (water and ethanol extracts)	Raji cells (human lymphoblastoid cell line	Water and ethanol extracts (40% and 96%) completely suppressed cell growth at 50–500 μg/ml	Spiridonov *et al.*, 1992
CAPE (caffeic acid phenethyl ester isolated from propolis)	Mouse C3H $10T\frac{1}{2}$; Mouse C3H $10T\frac{1}{2}$-BP[1]; Mouse C3H Ltk^-; Rat 6; T24-Rat 6; Monkey CV-1; Monkey Vero	˜10% of control growth at 2.5 μg CAPE/ml˜20% of control growth at 20 μg CAPE/ml˜5% of control growth at 10 μg CAPE/ml˜60% of control growth at 10–20 μg CAPE/ml˜20% of control growth at 20 μg CAPE/ml˜20% of control growth at 20 μg CAPE/ml˜10% of control growth at 20 μg CAPE/ml	Grundberger *et al.*, 1988
CAPE (caffeic acid phenethyl ester isolated from propolis)	CREF and transformed Wt 3A[2]	At 8 μg/ml 75% of CREF cells unaffected, while transformed cells 90% inhibited.	Grundberger *et al.*, 1988
CAPE (caffeic acid phenethyl ester isolated from propolis)	Human MCF-7 breast carcinoma	5 μg/ml CAPE inhibits incorporation of ^{3}H-thymidine incorporation by ˜50% and is completely blocked at 10 μg/ml.	Grundberger *et al.*, 1988
CAPE (caffeic acid phenethyl ester isolated from propolis)	Human SK-MEL-28 melanoma	Minimal incorporation at 5 μg CAPE/ml, complete inhibition at 10 μg/ml.	Grundberger *et al.*, 1988
CAPE (caffeic acid phenethyl ester isolated from propolis)	CREF and Ad5-transformed CREF cells	Growth suppression transformed by Ad5 or Ad5 E1A transforming gene, untransformed cells not affected.	Su *et al.*, 1991
CAPE (caffeic acid phenethyl ester isolated from propolis)	CREF and Ad5-transformed CREF cells	Only cells expressing the phenotype of Ad5 E1A and E1B transforming genes effected.	Su *et al.*, 1994
Methyl caffeate (MC), phenylethyl caffeate (PEC), phenylethyl dimethylcaffeate (PEDC)	Human colon cell line HT-29	MC $ID_{50} > 150$ μM; (↓) PTK = 100 μM; (↓) ODC = 150 μM PEC $ID_{50} = 55$ μM; (↓) PTK = 30 μM; (↓) ODC = 40 μM PEDC $ID_{50} = 36$ μM; (↓) PTK = 20 μM; (↓) ODC = 20 μM	Rao *et al.*, 1992
Methyl caffeate (MC), phenylethyl caffeate (PEC), phenylethyl dimethylcaffeate (PEDC)	Human colon HCT-116 (malignant type	PEC and PEDMC $ID_{50} < 25$ μM	Rao *et al.*, 1992
Clerodane diterpenoid (isolated from propolis)	Human hepatocellular carcinoma HuH 13 cells; Human lung carcinoma HLC-2; HeLa, KB and rat W3Y cells, untransformed rabbit kidney cells, human diploid cells	Growth of HuH 13 cells inhibited in S phase at 10 μg/ml, lethal at 20 μg/ml; lethal to HLC-2 cells at 50 μg/ml; cytotoxic to other cells at undisclosed concentrations; little cytotoxicity on confluent monolayers of untransformed primary rabbit kidney cells or human diploid cells.	Matsuno, 1995
Clerodane diterpenoid (isolated from propolis)	Human diploid foreskin, primary rabbit kidney cells	>75% survival at 100 μg/ml.	Matsuno, 1995

[1]Benzo[*a*]pyrene-transformed cells.
[2]Cloned cell line of Fischer rat embryo fibroblasts (CREF) and its counterpart, transformed by adenovirus serotype 5 (Wt3A).
(↓) = decreased.

of Peru, were negative to cinnamic acid, grass pollens and trees including poplar and others known to be sources of propolis.

DeGroot *et al.* (1994) have maintained the primary allergen in propolis as being 'LB-1', and consisting of a mixture of 3-methyl-2-butenyl caffeate (54%), 3-methyl-3-butenyl caffeate (28%), 2-methyl-2-butenyl caffeate (4%), phenylethyl caffeate (8%), caffeic acid (1%) and benzyl caffeate (1%). The majority-held opinion is, however, that LB-1 is 1,1-dimethylallyl caffeic acid ester (Acciai *et al.*, 1990; Bjorkner, 1994; Hausen *et al.*, 1987a,b).

In their work, Hausen *et al.* (1987b) attempted to identify the specific allergen and to determine whether there was a true cross-reaction or a pseudo cross-reaction. These investigators first isolated 1,1-dimethylallylcaffeic acid (LB-1) ester from the buds of *Populus nigra* L. They determined the threshold of irritation in guinea pigs via open epicutaneous application of three different dilutions of propolis (20%, 10%, 1%), and (LB-1) (10%, 3%, 1%) dissolved in acetone onto the clipped and shaved flank of guinea pigs. They report the threshold of irritation for propolis was found to be higher than 20% and for LB-1 to between 3 and 10% (Hausen *et al.*, 1987b). To determine sensitivity and specificity, the animals were given propolis or LB-1 in Freund's Complete Adjuvant. Challenge was 11 days after induction using open epicutaneous elicitation by application of 0.05 ml of subirritant doses of propolis and LB-1 on the clipped and shaved flanks of the sensitized animals. The reactions were read at 24, 48 and 72 hr. The results clearly demonstrated that propolis and its constituent LB-1 are both strong contact sensitizers. At a 1% concentration, the mean response of propolis was 2.6 and of LB-1 3.0 at the 72 hr reading. Challenge with LB-1 and poplar bud extracts on propolis-sensitive guinea pigs produced reactions as well and were as strong as propolis itself. The authors reasoned that these responses could not be regarded as 'cross-reactions' as the responsible sensitizer, LB-1, found in propolis, is a constituent derived from the poplar buds. Thus, in most cases of propolis allergy, the poplar bud constituent 1,1-dimethylallyl caffeic acid ester, must be considered as the responsible agent. (Hausen *et al.*, 1987b).

To determine the effect in humans, nine patients who were sensitive to propolis were patch tested with propolis (10% in white petrolatum), poplar bud extracts (1%), and LB-1 (1%). In some cases the flavonoid tectochrysin was tested (1% in petrolatum) as well. Application was carried out on the backs of the patients and read after 24 and 72 hr. In eight out of nine patients, LB-1 was positive at 2+ or greater at 72 hr. Balsam of Peru, included in the standard series, only gave a positive response in two out of nine. Positive reaction to tectochrysin (from poplar buds) was seen in three out of five patients (Hausen *et al.*, 1987a).

Acciai *et al.* (1990) synthesized dimethylallyl ester of caffeic acid (I) together with its *o*-methyl derivatives, with partially [dimethylallyl ester of ferulic acid (II)] or completely (dimethylallyl ester of 3,4-dimethoxycinnamic acid (III)) blocked hydroxyl functions. Thin-layer chromatography of poplar bud extract and propolis samples showed the presence of the first two compounds, but not the third. The authors patch tested nine subjects sensitized to propolis and other related allergens with compounds I, II and III. Three subjects reacted to I. Compounds II and III gave negative reactions in all cases. These findings confirm that dimethylallyl ester of caffeic acid has haptenic activity and lend support to the hypothesis that its sensitizing property may be related to the presence of free hydroxyl groups on the aromatic ring (Acciai *et al.*, 1990).

Whatever the primary allergen(s) might be, there are a number of substances with which the patients both 'pseudo cross-react' or cross-react. Pseudo cross-reactions are most commonly to balsams Peru and Tolu, the common constituents of which are benzoic acid, benzyl alcohol, benzyl benzoate, benzyl cinnamate, benzyl ferulate, farnesol, benzyl isoferulate, caffeic acid, cinnamic alcohol cinnamic acid, coniferyl benzoate, nerolidol and vanillin (DeGroot *et al.*, 1994). Whether there is any cross- or pseudo cross-reaction to one or any of these substances is debatable since both circumstances have been reported (Table 6).

At least a part of the key to the question of pseudo cross-reactivity to propolis, may lie in the immunostimulatory effects of propolis reported by a number of investigators. For example, Kivalkina and Budarkova (1975) reported that propolis, when injected simultaneously with concentrated tetanus anatoxin, either once or under conditions for hyperimmunization, stimulated non-specific and specific immunity factors and increased the preventive properties of immunizing sera and the resistance of animals to tetanus toxin. Budarkova (1976) also noted this effect using hyperimmunized rabbits to study the influence of propolis on the antigen properties of tetanus toxin adsorbed on aluminium hydroxide. The addition of an alcohol extract of propolis to tetanus toxin, at the rate of 5 mg dry weight per 20 units of toxin, enhanced the production of antitoxin with a series of injections, ranging from 20 units to 240 units of tetanus toxin, made at weekly intervals for 63 days (Budarkova, 1971). To define this reaction, Budarkova (1976) injected rabbits with 60 units of tetanus toxin, either with or without an alcohol extract of propolis (5 mg dry weight per ml antigen). The rabbits were killed at intervals of a few days up to 35 days after the injection. The investigators noted that maximum counts of plasmacytes in lymph nodes occurred 7 days after the injections; counts were always higher in rabbits injected with propolis than in the controls. The average initial count in a lymph node near the site of injection was 11; the average count in controls was 16, 28 and 13 after 4, 7 and 35 days, respectively, and in propolis-treated rabbits 21, 66 and 17. Similar but less pronounced changes were observed in lymph nodes further from the site of the injection (Budarkova, 1976).

Enhancement of the immune response does not appear, however, to be through an enhanced activation of complement. That is, when human or guinea pig complement is treated with a water-soluble

Table 6. Clinical reports of propolis allergy

Patient	Sensitivity to propolis	Sensitivity to other substances	Comment	Reference
22 Patients with dermatitis (associated with) propolis	21/22	19/22-balsam of Peru; sensitivity to clove oil and other essential oils	Of those sensitive to balsam of Peru, sensitivity was exhibited to other substances: cinnamyl cinnamate, vanillin and benzyl cinnamate	Rudzki and Grzywa, 1983
Violin maker sensitive to propolis-based varnish	Propolis and EtOH extract	Negative to polyester resin, cinnamic acid and aldehyde, colophony and balsam of Peru		Monti *et al.*, 1983
Woman with dermatitis after using a propolis-based face cream	Propolis and EtOH extract	Negative to polyester resin, cinnamic acid and aldehyde, colophony (resin) and balsam of Peru		Monti *et al.*, 1983
Part-time beekeeper with dermatitis on trunk and limbs and perennial cough when beekeeping in season	Patch test (+ +)	Balsam of Peru, house dust and wood		Cirasino *et al.*, 1987
55-Yr old female beekeeper	Patch test strongly positive (20% and 5% in petrolatum)	Allergic to balsam of Peru, negative to benzyl benzoate, benzoic acid, benzyl alcohol, benzyl cinnamate, coumarin, eugenol, isoeugenol, limonene, balsam of Tolu, vanillin, geraniol, lavender oil, cinnamic alcohol, cinnamic acid, cinnamic aldehyde, oil of cinnamon, styrax		Young, 1987
Five females who reacted to topical compounds containing propolis	Patch test (+ + +)	Also sensitive to beeswax and balsam of Peru; negative to cinnamic acid, grass pollens and trees including poplar	Authors suggest beeswax is contaminated with propolis; reaction to balsam of Peru is a pseudo-cross reaction.	Valsecchi and Cainelli, 1984
55 Yr-old accordion repairer	Propolis	Also positive to balsam of Peru and to beeswax.	Cross-contamination of beeswax with propolis?	Van Ketel and Bruynzeel, 1992
55-Yr-old man with stomatitis and throat complaints for some years. Patient had used tablets and toothpaste containing propolis	Propolis (strongly positive)	Also sensitive to balsam of Peru	Complaints stopped after cessation of use of propolis products.	Young, 1987
A 37-yr-old man had an episode of palmo-plantar dyshydrosis 1 year ago. A mild relapse occurred 1 month ago, which he treated with propolis both orally and locally. For about 10 days, he chewed a small piece of propolis once a day. Stomatitis was noted.	Propolis (20% in pet)	Also sensitive to beeswax and cinnamic acid. Negative to balsam of Peru.		Ayala *et al.*, 1985
2-yr-old male dog weighing about 45 kg. For 1 year it had had eczema in the sacro-lumbar region, which was rubbed with propolis alcohol solution	Propolis	Also sensitive to 25% balsam of Peru.	Tests were positive to intradermal to flea antigen	Rudzki *et al.*, 1985

derivative (WSD) of propolis *in vitro*, C3 functional activity is impaired, as indicated by suppression of complement-mediated haemolysis (Ivanovska *et al*., 1995b). This suppression of immune (antiinflammatory response) was also seen *in vivo* with mice when administered 150 mg/kg of WSD, intravenously or intraperitoneally (Ivanovska *et al*., 1995a). These investigators measured change in paw oedema 0.5, 2, 4 and 24 hr following zymosan injection. The WSD also influenced the process of acute inflammation provoked by zymosan in mice, regardless of route, although there was a delay in onset of difference when given by the ip route.

Miscellaneous activities. In addition to the antiinflammatory powers described above, Frenkel *et al*. (1993) noted that 12-*o*-tetradecanoylphorbol-13-acetate-induced hydrogen peroxide production in bovine lenses also is inhibited by CAPE. Cumulatively, their findings point to CAPE as being a potent chemopreventive agent, which may be useful in combating diseases with strong inflammatory and/or oxidative stress components, namely various types of cancer and possibly cataract development (Frenkel *et al*., 1993).

Stojko *et al*. (1978) noted that EEP accelerated wound healing of artificially lesioned bones of dogs. Scheller *et al*. (1977) noted a similar effect in the acceleration of regeneration processes in the lesioned cartilage. Further, EEP inserted into the joint is well tolerated (Scheller *et al*., 1977).

Summary

Propolis (sometimes also referred to 'bee glue') is the generic name for the resinous substance collected by honeybees from various plant sources. This resin is masticated, salivary enzymes added and the partially digested material is mixed with beeswax and used in the hive. As produced by the bees, propolis is a strongly adhesive, resinous substance used by bees to seal holes in their hives, smooth out the internal walls and protect the entrance against intruders.

Although a common source of the resin is *Populus balsamifera* L. (and other *Populus* species), the precise composition of raw propolis varies with the source. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris. Raw propolis is processed using water washing and solubilizing in 95% ethanol to remove the wax and organic debris, creating propolis tincture, 'propolis balsam', or ethanol extract of propolis (EEP).

Man's long history of bee domestication has led to a thorough exploitation of bee products, and the many favourable properties of both raw and refined propolis lend to its application in many human pursuits. Recorded use of propolis dates to at least to 300 BC and continues today in topical home remedies and personal products, as well as an ingredient in toothpaste and dental floss (1–5% of the finished product), and as a health-food/dietary supplement (recommended dosage, 200 mg/day). Current sales of propolis in the United States are estimated at 40,000 lb/yr. Propolis is also found in beeswax and extracted honey as an unintentional additive.

Although ethanol extract of propolis (EEP) is the most common, extracts with other solvents have been produced for identification of constituents. The largest group of compounds isolated from propolis tincture are flavonoid pigments, which are ubiquitous in the plant kingdom and the series of flavonoids isolated from propolis correlate reasonably well with those present in the plants from which honeybees collect propolis. The substances identified in propolis are familiar constituents of food, food additives and/or GRAS substances. Conspicuous among the list of constituents are hydroquinone, caffeic acid (and its esters), and quercetin, each of which have exhibited carcinogenic effects when administered to rodents.

Propolis has a low order of acute oral toxicity with reported LD_{50} ranging from 2000 to 7300 mg/kg in mice. Flavonoids, the primary constituents of propolis, are reported to have oral LD_{50} in rats of 8000–40,000 mg/kg. The threshold of irritation in guinea pigs was not achieved with a 20% solution of propolis in acetone and propolis applied neat or in ointment was not irritating to rabbits.

Propolis, administered orally to mice at levels up to 4000 mg/kg/day for 2 wk had no effect. 90 days of administration to mice in drinking water at 1400 mg/kg/day was declared a NOEL and a 60-day drinking water study in rats demonstrated no propolis-related effects at 2470 mg/kg/day.

Propolis and its constituent flavanoids exhibit an antitumor effect both *in vivo* and *in vitro*. It is cytotoxic or cytostatic to several yeasts, molds, bacteria and parasites *in vivo* and/or *in vitro*.

Propolis and has been identified clinically as an allergen and is reported to have immunological stimulating properties consistent with its allergenic characteristics.

If the NOEL in mice of 1400 mg/kg/day is applied to human safety, a safety factor of 1000 should be employed to account for a lack of chronic toxicity studies. Therefore a safe dose in humans would be 1.4 mg/kg body weight/day, or approximately 70 mg/day.

REFERENCES

Abdou R. F. and Omar M. O. M. (1988) Effect of the bee product 'propolis' on the mitotic cells of *Vicia faba*, *Hordeum vulgare, Allium cepa* and *Allium sativum*. *Assiut Journal of Agricultural Sciences* **19,** 159–170.

Acciai M. C., Ginanneschi M., Bracci S. and Sertoli A. (1990) Studies of the sensitizing properties of propolis. *Contact Dermatitis* **23,** 274–275.

Amoros M., Sauvager F., Girre L. and Cormier M. (1992) *In vitro* antiviral activity of propolis. *Apidologie* **23,** 231–240.

Arctander S. (1960) *Perfume and Flavor Materials of Natural Origin*. pp. 736. S. Arctander, Elizabeth, NJ.

Arvouet-Grand A., Lejeune B., Bastide P., Pourrat A. and Legret P. *et al*. (1993) Propolis extract. Part 6. Subacute toxicity and cutaneous primary irritation index. *Journal de Pharmacie de Belgique* **48,** 165–170.

Ayala F., Lembo G., Nappa P. and Balato N. (1985) Contact dermatitis from propolis. *Contact Dermatitis* **12,** 181–182.

Ban J., Popovic S. and Maysinger D. (1983) Cytostatic effects of propolis *in vitro*. *Acta Pharmaceutica Jugoslavica* **33,** 245–255.

Bankova V., Christov R., Kujumgiev A., Marcucci M. C. and Popov S. (1995) Chemical composition and antibacterial activity of Brazilian propolis. *Zeitschrift für Naturforschung Section C. Biosciences* **50,** 167–172.

Bankova V., Christov R., Popov S., Pureb O. and Bocari G. (1994) Volatile constituents of propolis. *Zeitschrift für Naturforschung Section C. Biosciences* **49,** 6–10.

Bankova V. S., Popov S. S. and Marekov N. L. (1983) A study on flavonoids of propolis. *Journal of Natural Products* **46,** 471–474.

Bisson C. S. (1940) Investigations on the physical and chemical properties of beeswax. United States Department of Agriculture. Technical Bulletin No. 716. pp. 1–24.

Bjorkner B. E. (1994) Industrial airborne dermatoses. *Dermatology Clinics* **12,** 501–509.

Budarkova E. L. (1971) Effect of propolis on immunological indices in tests of hyperimmunization with tetanus anatoxin. *Uchenye Zapiski Kazanskovo Veterinarnogo Instituta* **109,** 161–164.

Budarkova E. L. (1976) Cellular and humoral reactions to tetanus toxoid plus propolis. *Uchenye Zapiski Kazanskogo Gosudarstvennogo Veterinarnogo Instituta* **117,** 148–151.

Burdock G. A. (1995) *Fenaroli's Handbook of Flavor Ingredients*. Vol. I. 3rd Ed. pp. 350. CRC Press, Boca Raton, FL.

CHEMID (1996) A chemical database sponsored by the National Library of Medicine. Bethesda, MD.

Chiao C., Carothers A. M., Grunberger D., Solomon G., Presto G. A. and Barrett J. C. D. (1995) Apoptosis and altered redox state induced by caffeic acid phenethyl ester (CAPE) in transformed rat fibroblast cells. *Cancer Research* **55,** 3576–3583.

Cirasino L., Pisati A. and Fasani F. (1987) Contact dermatitis from propolis. *Contact Dermatitis* **16,** 110–111.

Coprean D., Rusu M., Giurgea R., Popescu H. and Polinicencu C. (1986) Effect of standardized propolis extract in the experimentally intoxicated liver of rats. [Efectul tratamentului cu extract ce propolis standardizat (EPS) in intoxicarea experimentala a ficatului la sbolan]. *Clujul Medical* **59,** 333–337.

Debiaggi M., Tateo F., Pagani L., Luini M. and Romero E. (1990) Effects of propolis flavonoids on virus infectivity and replication. *Microbiologica (Italy)* **13,** 207–213.

DeCastro S. L. and Higashi K. O. (1995) Effect of different formulations of propolis on mice infected with *Trypanosoma cruzi*. *Journal of Ethnopharmacology* **46,** 55–58.

DeGroot A. C., Weyland J. W. and Nater J. P. (1994) *Unwanted Effects of Cosmetics and Drugs Used in Dermatology*. 3rd Ed. pp. 770. Elsevier, New York.

Digrak M., Yilmaz O. and Ozcelik S. (1995) *In vitro* antimicrobial effect of propolis collected in Elazig region. *Turkish Journal of Biology* **19,** 249–257.

Dobrowolski J. W., Vohora S. B., Sharma K., Shah S. A., Naqvi S. A. H. and Dandiya P. C. (1991) Antibacterial, antifungal, antiamoebic, antiinflammatory and antipyretic studies on propolis bee products. *Journal of Ethnopharmacology* **35,** 77–82.

Drogovoz S. M., Tikhonov A. I., Slyshkov V. V. and Salnikova S. I. (1994) Examining the hepatoprotective properties of pediatric propolis dosage form in animals of various age groups. *Experimental and Clinical Pharmacology* **57,** 39–42.

Esser B. (1986) Allergy due to propolis. *Aktuelle Dermatologie* **12,** 203–205.

Frenkel K., Wei H., Bhimani R., Ye J., Zadunaisky J. A., Huang M.-T., Ferraro T., Conney A. H. and Grunberger D. (1993) Inhibition of tumor promoter-mediated processes in mouse skin and bovine lens by caffeic acid phenethyl ester. *Cancer Research* **53,** 1255–1261.

Ghisalberti E. L. (1979) Propolis: a review. *Bee World* **60,** 59–84.

Gonzalez R., Remirez D., Rodriguez S., Gonzalez A., Ancheta O., Merino N. and Pascual C. (1994) Hepatoprotective effects of propolis extract on paracetamol-induced liver damage in mice. *Phytotherapy Research* **8,** 229–232.

Gonzalez R., Corcho I., Remirez D., Rodriguez S., Ancheta O., Merino N., Gonzalez A. and Pascual C. (1995) Hepatoprotective effects of propolis extract on carbon tetrachloride-induced liver injury in rats. *Phytotherapy Research* **9,** 114–117.

Greenaway W., May J., Scaysbrook T. and Whatley F. R. (1991) Identification by gas chromatography-mass spectrometry of 150 compounds in propolis. *Zeitschrift für Naturforschung* **46c,** 111–121.

Greenaway W., Scaysbrook T. and Whatley F. R. (1987) The analysis of bud exudate of *Poppulus x euramericana*, and of propolis, by gas chromatography-mass spectrometry. *Proceedings of the Royal Society, London B* **232,** 249–272.

Greenaway W., Scaysbrook T. and Whatley F. R. (1990) The composition and plant origins of propolis: a report of work at Oxford. *Bee World* **71,** 107–118.

Grundberger D., Banerjee R., Eisinger K., Oltz E. M., Efros L., Caldwell M., Estevez V. and Nakanishi K. (1988) Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. *Experientia* **44,** 230–232.

Han S. K. and Park H. K. (1995) A study on the preservation of meat products by natural propolis: effect of EEP on protein change of meat products. *Korean Journal of Animal Science* **37,** 551–557.

Hausen B. M., Wollenweber E., Senff H. and Post B. (1987a) Propolis allergy I. Origin properties usage and literature review. *Contact Dermatitis* **17,** 163–170.

Hausen B. M., Wollenweber E., Senff H. and Post B. (1987b) Propolis allergy II. The sensitizing properties of 1,1-dimethylallyl caffeic acid ester. *Contact Dermatitis* **17,** 163–170.

Havsteen B. (1983) Flavonoids, a class of natural products of high pharmacological potency. *Biochemical Pharmacology* **32,** 1141–1148.

Hladon B., Bylka W., Ellnain-Wojtaszek M., Skrzypczak L., Szafarek P., Chodera A. and Kowalewski Z. (1980) *In vitro* studies on the cytostatic activity of propolis extracts. *Arzneimettel-Forschung* **30,** 1847–1848.

Hollands I., Vidal A., Gra B. and Sotolongo M. (1991) Evaluation of the sub-chronic toxicity of Cuban propolis. [Estudio evaluativo do la toxicidad subcronica del propoleos cubano]. *Revista Cubana de Ciencias Veterinarias* **22,** 91–100.

Hrytsenko V. I., Tykhonov O. I. and Pryakhin O. R. (1977) Study of the polysaccharide preparation propolis. *Farmatsevtychnyi Zhurnal* **32,** 92–93.

IARC (1983) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 31. Quercetin*. pp.

213–229. International Agency for Research on Cancer, Lyon.

IARC (1993) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 56. Caffeic acid.* pp. 115–134. International Agency for Research on Cancer, Lyon.

Ikeno K., Ikeno T. and Miyazawa C. (1991) Effects of propolis on dental caries in rats. *Caries Research* **25,** 347–351.

Ivanovska N. D., Dimov V. D., Bankova V. S. and Popov S. S. (1995a) Immunomodulatory action of propolis. VI. Influence of a water soluble derivative on complement activity *in vivo*. *Journal of Ethnopharmacology* **47,** 145–147.

Ivanovska N. D., Dimov V. B., Pavlova S., Bankova V. S. and Popov S. S. (1995b) Immunomodulatory action of propolis. V. Anticomplementary activity of a water-soluble derivative. *Journal of Ethnopharmacology* **47,** 135–143.

Johnson K. S., Eischen F. A. and Giannasi D. E. (1994) Chemical composition of North American bee propolis and biological activity towards larvae of greater wax moth (Lepidoptera: Pyralidae). *Journal of Chemical Ecology* **20,** 1783–1792.

Kaneeda J. and Nishina T. (1994) Safety of propolis. Acute toxicity. *Honeybee Science* **15,** 29–33.

Kivalkina V. P. and Budarkova E. L. (1975) Effect of propolis on antigenic and immunolgenic properties of tetanic anatoxin. Fitontsidy: eksperimental'nye issledovaniya voprosy teorii I praktiki. Edited by B. E. Aizenman *et al*. pp. 269–270.

Kleinrok Z., Borzecki Z., Scheller S. and Matuga W. (1978) Biological properties and clinical application of propolis. X. Preliminary pharmacological evaluation of ethanol extract of propolis (EEP). *Arzneimittel-Forschung* **28,** 291–292.

Koltay M. (1981) Isolation and identification of constituents of propolis. MSc Thesis. Concordia University, Montreal, Quebec, Canada.

Lisowski F. (1984) Demystifying health foods. *On Continuing Practice* **11,** 11–14.

Maksimova-Todorova V., Manolova N., Gegova G., Serkedzhieva Yu., Uzunov S., Pancheva S., Marekov N. and Bankova V. (1985) Antiviral effects of some fractions isolated from propolis. *Acta Microbiologica Bulgarica* **17,** 79–85.

Marcucci M. C. (1995) Propolis: Chemical composition, biological properties and therapeutic activity. *Apidologie* **26,** 83–99.

Matsuno T. (1995) A new clerodane diterpenoid isolated from propolis. *Zeitschrift für Naturforschung Section C. Biosciences* **50,** 93–97.

Metzner J., Schneidewind E.-M. and Friedrich E. (1977) Effects of propolis and pinocembrin on yeasts. [Zur Wirkung von Propolis und Pinocembrin auf Sprosspilze]. *Pharmazie* **32,** 730.

Meyer W. (1956) Propolis bees and their activities. *Bee World* **37,** 25–36.

Monti M., Berti E., Carminati G. and Cusini M. (1983) Occupational and cosmetic dermatitis from propolis. *Contact Dermatitis* **9,** 163.

NTP (1992) Toxicology and carcinogenesis studies of quercetin in F344/N rats (feed studies). National Toxicology Program, Research Triangle Park, NC. TRS 409. NTIS PB93-147478.

Pepeljnjak S., Jalsenjak I. and Maysinger D. (1982) Inhibition of growth and biosynthesis of ochratoxin A in *Aspergillus sulphureus* NRRL 4077 by propolis extract. *Pharmazie* **37,** 439–440.

Pepeljnjak S., Jalsenjak I. and Maysinger D. (1985) Flavonoid content in propolis extracts and growth inhibition of *Bacillus subtilis*. *Pharmazie* **40,** 122–123.

Rao C. V., Desai D., Kaul B., Amin S. and Reddy B. S. (1992) Effect of caffeic acid esters on carcinogen-induced mutagenicity and human colon adenocarcinoma cell growth. *Chemico-Biological Interactions* **84,** 277–290.

Rao C. V., Desai D., Rivenson A., Simi B., Amin S. and Reddy B. S. (1995) Chemoprevention of colon carcinogenesis by phenylethyl-3-methylcaffeate. *Cancer Research* **55,** 2310–2315.

Rao C. V., Desai D., Simi B., Kulkarni N., Amin S. and Reddy B. S. (1993) Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant crypt foci formation in rat colon. *Cancer Research* **53,** 4182–4188.

Rojas Hernandez N. M., Candelario M. and Olivares E. (1993) Antimicrobial activity of propolis against representatives of the genus *Mycobacterium*. *Revista Biologia (Habana)* **7,** 69–75.

Rudzki E. and Grzywa Z. (1983) Dermatitis from propolis. *Contact Dermatitis* **9,** 40–45.

Rudzki E., Grzywa Z. and Pomorski Z. (1985) New data on dermatitis from propolis. *Contact Dermatitis* **13,** 198–199.

Scheller S., Krol W., Swiacik J., Owczarek S., Gabrys S. and Shani J. (1989) Antitumoral property of ethanolic extract of propolis in mice-bearing Ehrlich carcinoma, as compared to bleomycin. *Zeitschrift für Naturforschung C* **44,** 1063–1065.

Scheller S., Stojko A., Szwarnowiecka I., Tustanowski J. and Obuszko Z. (1977) Biological properties and clinical application of propolis. VI. Investigation of the influence of ethanol extracts of propolis (EEP) on cartilaginous tissue regeneration. *Arzneimittel Forschung* **27,** 2138–2140.

Schevchenko, L.F., Chasovodtseva, O.A. and Peschanskii, A.N. (1972) Inhibiting activity of propolis on the influenza virus. Conference title: Khimioprofilakika I Khimioterapiya Grippa, Materialy Vsesoyuznogo Simpoziuma po Khimioprofilaktike I Khimioterapil Grippa, 1st 1971. Edited by A.A. Smorodintsev and D.M. Vlydnikov. pp. 56–57.

SCOGS (1975) Evaluation of the health aspects of beeswax (yellow or white) as a food ingredient. Select Committee on GRAS Substances. Life Sciences Research Office. Federation of American Societies for Experimental Biology, Bethesda, MD.

Serkedjieva J., Manolova N. and Bankova V. (1992) Anti-influenza virus effect of some propolis constituents and their analogues (esters of substituted cinnamic acids). *Journal of Natural Products Lloydia* **55,** 294–297.

Spiridonov N. A., Arkhipov V. V., Narimanov A. A., Shabalina S. A., Zverkova L. A., Shvirst E. M. and Kondrashova M. N. (1992) Effect of *Galleria mellonella* larvae preparation and honeybee products on cell cultures. *Comparative Biochemistry and Physiology C Comparative Pharmacology and Toxicology* **102,** 205–208.

Starzyk J., Scheller S., Szaflarski J., Moskwa M. and Stojko A. (1977) Biological properties find clinical application of propolis. II. Studies on the on the antiprotozoan activity of ethanol extract of propolis. *Arzneimittel-Forschung* **27,** 1198–1199.

Stojko A., Scheller S., Szwarnowiecka I., Tustanowski J., Ostach H. and Obuszko Z. (1978) Biological properties and clinical application of propolis. VII. Experimental observations on the influence of ethanol extract of propolis (EEP) on the regeneration of bone tissue. *Arzneimittel-Forschung* **28,** 35–37.

Su Z.-Z., Grunberger D. and Fisher P. B. (1991) Suppression of adenovirus type 5 E1A-mediated transformation and expression of the transformed phenotype by caffeic acid phenethyl ester (CAPE). *Molecular Carcinogenesis* **4,** 231–242.

Su Z.-Z., Lin J., Grunberger D. and Fisher P. B. (1994) Growth suppression and toxicity induced by caffeic acid phenethyl ester (CAPE) in type 5 adenovirus-transformed rat embryo cells correlate directly with transformation progression. *Cancer Research* **54,** 1865–1870.

USDA (1985) United States Department of Agriculture. United States Standards for Grades of Extracted Honey. Agricultural Marketing Service. Fruit and Vegetable Division. Processed Products Branch. Washington, DC.

Valsecchi R. and Cainelli T. (1984) Dermatitis from propolis. *Contact Dermatitis* **11,** 317.

Van Ketel W. G. and Bruynzeel D. P. (1992) Occupational dermatitis in an accordion repairer. *Contact Dermatitis* **27,** 186.

Webster L. T. (1990) Drugs used in the chemotherapy of protozoal infections. In *The Pharmacological Basis of Therapeutics*. 8th Ed. Edited by A. G. Gilman, T. W. Rall, A. S. Nies and P. Tayler. pp. 999–1007. Pergamon Press, New York.

Young E. (1987) Sensitivity to propolis. *Contact Dermatitis* **16,** 49–50.