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Review

Pharmacognostic and pharmacological profile of *Humulus lupulus* L.

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Abstract

The present review describes the morphological, phytochemical and ethnopharmacological aspects of *Humulus lupulus* L. (Cannabinaceae) and summarizes the most interesting findings obtained in the preclinical and clinical research related to the plant. The female inflorescences of *Humulus lupulus* (hops), well-known as bittering agent in brewing industry, have long been used in traditional medicine mainly to treat sleep disturbances. However the sedative activity is still under investigation in order to recognize the active principles responsible for the neuropharmacological effects observed in laboratory animals, and their mechanism of action. Here we report the data from our experiments as well as those obtained from other researchers, focusing on the variability of the results. Other traditional applications of hops as stomachic, antibacterial and antifungal remedy have been supported by *in vivo* and/or *in vitro* investigations. In recent years some prenylated chalcones present in hops have received much attention for their biological effects: in particular, xanthohumol has been shown to exert cancer chemopreventive activity in *in vitro* experiments, while 8-prenylnaringenin has been characterized as one of the most potent phytoestrogens isolated until now. Nevertheless much additional work is needed to open up new biomedical application of these compounds.

Keywords: *Humulus lupulus*; Hops; Pharmacognosy; Pharmacological profile; Sedative activity

Contents

Abbreviations: 6-PN, 6-prenylnaringenin; 8-PN, 8-prenylnaringenin; A_1, adenosine receptor type 1; b.w., body weight; CNS, central nervous system; CO_2, carbon dioxide; DMX, desmethylxanthohumol; ER, estrogen receptor; ERE, estrogen responsive element; FSH, follicle stimulating hormone; GABA, γ-aminobutyric acid; ^{1}H NMR, proton nuclear magnetic resonance; HPLC, high performance liquid chromatography; 5-HT, 5-hydroxytriptamine; 5-HT_6, 5-hydroxytriptamine receptor type 6; IgE, immunoglobulin E; i.p., intraperitoneally; IX, isoxanthohumol; KS-IMM, immortalized Kaposi's sarcoma cell line; LD_{50}, half-maximal lethal dose; LH, luteinizing hormone; M., molar concentration; MIC, minimal inhibitory concentration; ML_1, melatoninergic receptor type 1; mRNA, messenger ribonucleic acid; PCR, polymerase chain reaction; spp., species; TST, tail skin temperature; var., variety; XG, xanthogalenol; XH, xanthohumol.

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1. Introduction

The plant of *Humulus lupulus* L. is well-known throughout the world as the raw material in the brewing industry. The female inflorescences (hop cones or "hops"), rich in polyphenolic compounds and acyl phloroglucides are widely used to preserve beer and to give it a characteristic aroma and flavour. In addition hop cones have long been used for medicinal purposes. In particular, hop preparations were mainly recommended for the treatment of sleeping disorders, as a mild sedative, and for the activation of gastric function as bitter stomachic.

In line with a growing interest in the health benefits of plants used in traditional medicine, *Humulus lupulus* has received particular attention by the researchers and, as a result, a significant number of articles have been published. Starting from the second half of the 20th century, several phytochemical studies were performed to investigate the composition of hop cones and other parts of the plant, leading to the isolation and identification of pharmacologically relevant compounds such as flavanones, chalcones, phloroglucinol derivatives. During the past decade, many pharmacological investigations *in vitro* and *in vivo* tried to produce scientific evidence of the reported traditional uses. The effect of hop plant at the central nervous system (CNS) level and in particular its efficacy in sleeping disturbances has been repetitively studied in laboratory animals, but the results are sometimes contradictory and require a through reinvestigation. Moreover the number of clinical studies supporting the use of hops as a sedative is rather limited: therefore the effectiveness of hops in the treatment of sleeplessness is still questionable.

In recent years the estrogenic properties as well as the potential cancer chemopreventive activities of hops have been investigated and some active compounds from hop have received much attention. Among these, 8-prenylnaringenin is considered as one of the most potent phytoestrogens currently known, while xanthohumol proved to possess a broad spectrum of cancer-inhibiting mechanisms.

Starting from the current knowledge about the traditional use of hops and its botanical, phytochemical and pharmacological characteristics, the present review provides a critical appraisal of the ethnopharmacological issues. Particularly we focused our attention on the effect of the hop plant on CNS, comparing the results obtained in our laboratory and already published in this journal (Zanoli et al., 2005, 2007) with those obtained by other authors. Other effects of the hop plant, such as the estrogenic and cancer-related bioactivities, are only briefly discussed since comprehensive reviews have been recently published (Stevens and Page, 2004; Gerhäuser, 2005a; Chadwick et al., 2006).

2. Phytogeography

The genus *Humulus*, belonging to the family of Cannabinaceae, consists of three species: *Humulus lupulus* Linneus, *Humulus japonicus* Siebold & Zucc. and *Humulus yunnanensis* Hu (Small, 1978; Neve, 1991). The origin of the genus has been suggested to be China, because all *Humulus* species were found in this area (Small, 1980; Neve, 1991; Murakami et al., 2006a). From China an eastward migration to Japan and America and a westward migration to Europe should be responsible for the actual distribution of *Humulus* species. *Humulus lupulus* (commonly named hops) is naturalized in central Europe and it is widely cultivated throughout the temperate regions in the world (North and South America, South Africa, Australia). The species *Humulus lupulus* has been classified by Small (1978) into five taxonomic varieties based on their morphological characteristics and geographical locations: the var. *lupulus* Small for European hops, the var. *cordifolius* Small for Japanese hops, the var. *neomexicanus* Nelson & Cockerell, the var. *pubescens* Small and the var. *lupuloides* Small for North American hops. The molecular phylogeny, based on the nucleotides of nuclear and chloroplast DNA, has demonstrated a clear difference among North American, Asian and European hops, with divergence of European hops from the others occurring approximately one million years ago, followed by the diversification of Asian and North American hops, which are genetically close (Murakami et al., 2006a,b). The cultivation of *Humulus lupulus* for long time caused the existence of hundreds of named cultivars and many recognized chemotypes (Neve, 1991). The major reason was the need to select specific organoleptic properties with the aim to improve the flavour and aroma of beer, besides to produce different bitterness levels (Chadwick et al., 2006). Therefore cultivars with an increased content in volatile oil or bitter acids have been selected (Burgess, 1964; Neve, 1991).

3. History and ethnopharmacology

As reported by Behre (1999), in Europe there are only a few single findings of *Humulus lupulus* from prehistoric periods, while there is an increased number of sites and quantities of findings from the early Middle Ages, probably due to an increased utilization of the plant in brewing process. The oldest sources of *Humulus lupulus* in Europe and its use in brewing were described by Wilson (1975). At the earliest the plants were collected in the wild. The cultivation of hops started from the middle of the ninth century, between A.D. 859 and 875, in Germany where it extended from north to south during the early and high Medieval period, as well as to other regions of central Europe. At the beginning *Humulus lupulus* was utilized as an alternative to *Myrica gale* which was the prevailing beer additive inside the European area where it was native. In the eighteenth century the use of *Humulus lupulus* overcame that of *Myrica gale* due to its better preserving property (Behre, 1999). Currently the beer brewing industry accounts for 98% of the world

use of hops. Originally *Humulus lupulus* was used as a preservative for its antimicrobial activity; later it was additionally used to add a bitter flavour to beer (Moir, 2000). Moreover it is able to stabilize beer foam mainly due to the highly hydrophobic components *iso*-α-acids (Simpson and Hughes, 1994); also xanthohumol and its derivative isoxanthohumol were found to have positive effects on foam stability (Smith et al., 1998; Wilson et al., 1998).

Humulus lupulus was firstly mentioned by the naturalist Pliny the Elder (23–79 A.D.) who described the use of the young shoots as a vegetable by the Romans (Grieve, 1971). The leaves and flower-heads were used to produce a fine brown dye (Grieve, 1971). The flowers are a natural source of food flavouring for cereals, spices, sauces, tobacco and alcoholic beverages other than beer (Lawless, 1995; Barnes et al., 2002). The fibrous stems, similarly to hemp (*Cannabis sativa*), were used in the manufacture of a coarse kind of cloths and in the production of paper (Grieve, 1971). Hops were used in perfumes, especially the spicy and oriental types, in skin creams and lotions (Lawless, 1995).

Humulus lupulus has a long history as a medicinal remedy to treat a wide range of complaints. It has been mainly recommended as a mild sedative useful to treat sleeplessness and nervousness (Blumenthal, 1998). Traditionally hops were used to treat excitability and restlessness associated to tension headache; to improve appetite and digestion; to relieve toothache, earache and neuralgia (Grieve, 1971; Barnes et al., 2002). In addition hops have been reputed to exert diuretic, antispasmodic and anaphrodisiac effects (Duke, 1985; Weiss, 1988; Blumenthal, 1998). Native American tribes used hops as a sedative, antirheumatic, analgesic and as a urinary aid for "gravel" and inflammation (Hamel and Chiltoskey, 1975; Blumenthal, 1998; Bown, 2001). Also they used heated hops as a poultice in the treatment of pneumonia (Carr and Westey, 1945) and a decoction of hops was recommended for intestinal pain and fevers in Dakota (Bown, 2001). In India, the Ayurvedic Pharmacopoeia recommends hops to treat restlessness associated with nervous tension, headache and indigestion (Karnick, 1994). In traditional Chinese medicine hops are used to treat insomnia, restlessness, dyspepsia and lack of appetite. Alcoholic extracts of hops have been clinically used in China to treat leprosy, pulmonary tuberculosis, acute bacterial dysentery, silicosis and asbestosis with positive outcomes (Blumenthal et al., 2000). Topically hops were used to treat crural ulcers and skin injuries and to relieve muscle spasms and nerve pain (Lawless, 1995; Tyler and Foster, 1999; Wichtl and Brinckmann, 2004). In aromatherapy hops have been used for skin care, breathing conditions, nervousness, nerve pain and stress-related conditions (Lawless, 1995).

The Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMEA) (2007) report the traditional use of *Humulus lupulus* flos for relief of mild symptoms of mental stress and insomnia. The German Commission E and European Scientific Cooperative on Phytotherapy (ESCOP, 2003) approved hops as a treatment for excitability, mood disturbances (restlessness, anxiety) and sleep disturbances (Blumenthal, 1998).

4. Botany

Humulus lupulus L. is a perennial plant which regrows each spring from the rhizomes of an underground rootstock. It is a vine producing stems annual, slender, climbing, growing up to 6–9 m in length, often with stout-hooked hairs (Burgess, 1964; Neve, 1991). The stems twist around their support in a clockwise direction. A reference to the plant's habit of climbing on other plants is reflected in its name *Lupulus*, which is derived from the latin term lupus, a wolf climbing on a sheep (Grieve, 1971). In addition the English common name hop comes from the Anglo-Saxon *hoppan* meaning to climb. The origin of the name *Humulus* is doubtful but it has been suggested to come from *humus*, the rich moist ground in which the plant grows.

The leaves are dark-green coloured, long petiolate, heart-shaped with 3–5 lobes, sharply toothed and they have a very rough surface. They are placed opposite one another on the stem, but sometimes the upper leaves are arranged singly on the stem. It is a dioecious plant with male and female flowers on separate plants, although individual monoecious plants are frequently found in some wild North American hop populations, instead rarely found among the European types (Haunold, 1991; Haunold et al., 1993). Male and female plants are easily distinguished for their different flowers; no other morphological differences identify the sex of the plant. The male flowers are long racemes, 7.5–12.5 cm long, while the female inflorescences are cone-like catkins (called strobiles), 2.5–5 cm long, made up of overlapping membranaceous bracts. The external bracts are flattened and symmetrical. The internal bracts are longer and generally enfolding at the base a small fruit (achene). A resinous substance, named lupulin, is secreted by yellow glandular trichomes found at the base of cone bracts and can be separated by shaking the strobiles. Lupulin-like glands are also present on the underside of hop leaves. Female strobiles are collected in August–September, when they are ripe and their colour changes from pale greenish-yellow to yellow-brown. Only female individuals are present in hop-growing areas in order to maintain a genetically consistent product (Neve, 1991). Males are essential, however, in hop breeding programs to develop new varieties through controlled hybridization.

5. Phytochemistry

The main structural classes of chemical compounds identified from hop mature cones include terpenes, bitter acids and chalcones. Hops are also rich in flavonol glycosides (kaempferol, quercetin, quercitrin, rutin) (Sägesser and Deinzer, 1996) and catechins (catechin gallate, epicatechin gallate) (Gorissen et al., 1968).

Hundreds of terpenoid components were identified in the volatile oil (0.3–1.0% of hop strobile weight): primarily β-caryophyllene, farnesene and humulene (sesquiterpenes) and myrcene (monoterpene) (Malizia et al., 1999; Eri et al., 2000).

The bitter acids (5–20% of hop strobile weight) are phloroglucinol derivatives usually classified as α-acids and β-acids. Both groups contain a 3-,4-,5-, or 6-carbon oxo-alkyl side chain: β-acids are structurally different from α-acids for one

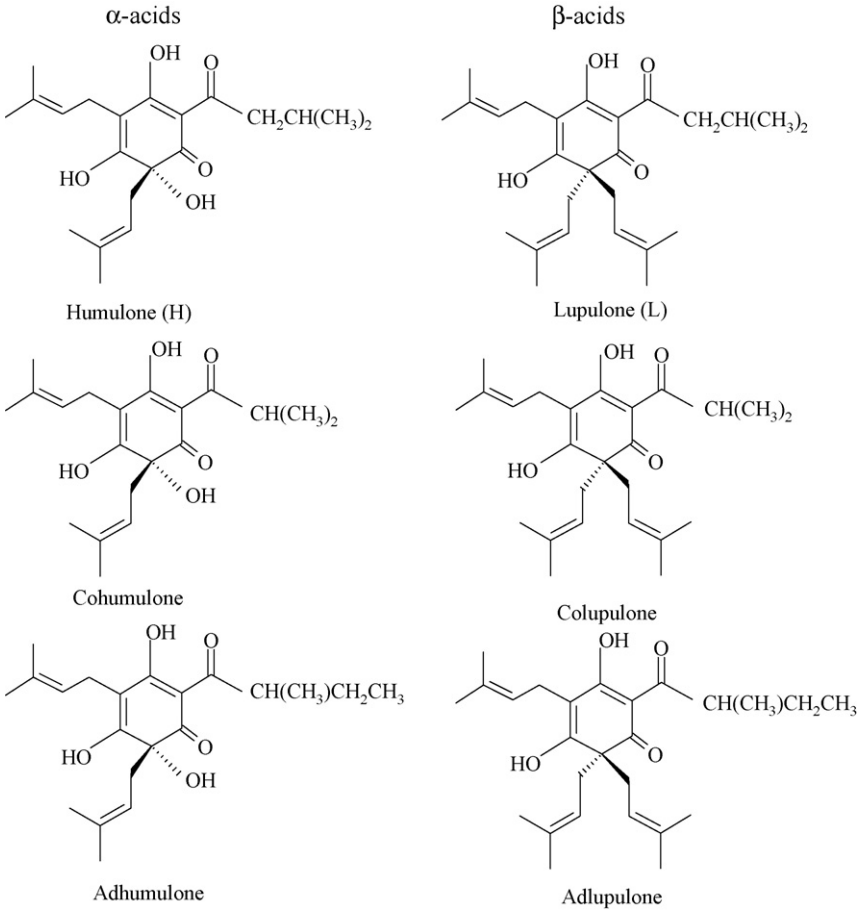

Fig. 1. Chemical structures of hop bitter acids.

more prenyl group. The bitter acids are present in hops as a complex mixture of variable composition and concentrations. The main α-acids are humulone (35–70% of total α-acids), cohumulone (20–65%) and adhumulone (10–15%); the corresponding β-acids are lupulone (30–55% of total β-acids), colupulone and adlupulone (Fig. 1). In addition to the two series of normal, co- and ad-homologs, there exist some minor bitter acids represented by posthumulone/postlupulone, prehumulone/prelupulone, adprehumulone. The biosynthesis, isomerization, oxidation and degradation of hop bitter acids have been extensively studied (Verzele and De Keukeleire, 1991; Fung et al., 1997; Goese et al., 1999). α-Acids are the crucial compounds for the quality of hops used in brewer industry, contributing to foam stability as well as exerting antibacterial activity (Verzele and De Keukeleire, 1991). At high pH value and high temperature, α-acids isomerize to the corresponding *iso*-α-acids which are more soluble and more bitter than their parent compounds. Therefore they are responsible mainly for the typical bitter taste of beer, in addition to elicit foam-stabilizing and antibacterial properties, like α-acids (Verzele and De Keukeleire, 1991).

Besides to the volatile oil and the bitter acids, several prenylflavonoids were identified from hop cones (Stevens et al., 1997) (Fig. 2). The most important compound is the chalcone xanthohumol (XH) (up to 1% in dry hop cones) (Stevens et al., 1999), which can be converted to the prenylflavanone isoxanthohumol (IX) in consequence of thermal treatment and increased pH value (Stevens et al., 1998, 1999). Therefore IX is the main prenylflavonoid present in beer. Also other chalcones, occurring at 10–100-fold lower concentrations than that of XH, isomerize to the corresponding flavanones. A chalcone named xanthogalenol (XG) has been identified only in some hop varieties growing in North America or East Asia (Stevens et al., 2000). The compound 2′,4′,6′,4-tetrahydroxy-3′-C-prenylchalcone commonly known as desmethylxanthohumol (DMX) is considered as the precursor of the most flavonoids present in hops (Chadwick et al., 2006). Through a chemical isomerization, it gives rise to the major estrogen of hops identified as the 1:1 racemate (±)-8-prenylnaringenin (8-PN), along with the racemic 6-prenylnaringenin (6-PN) (Hänsel and Schulz, 1988). In humans 8-PN has been shown to derive from IX through activation by intestinal microflora (Possemiers et al., 2006) or by liver cytochrome P450 enzymes (Guo et al., 2006). Hence, the estrogenically inactive XH possesses an estrogenic potential through its conversion to IX and then to 8-PN.

The chemistry, biological activity and biotechnological aspects of xanthohumol and other prenylated flavonoids from hops have been recently reviewed (Stevens and Page, 2004).

During the development from female inflorescences to ripe cones, the levels of α-acids, β-acids, DMX and XH gradually increase, the accumulation rate depending on hop variety and climatological conditions (De Keukeleire et al., 2003, 2007).

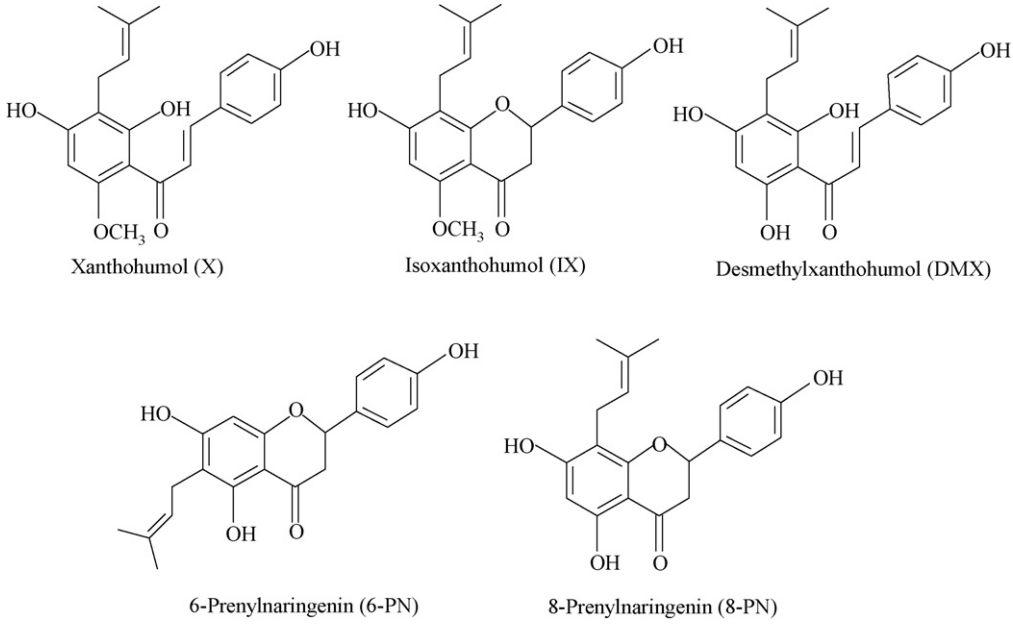

Fig. 2. Chemical structures of prenylflavonoids present in hops.

The bitter acids and XH were also detected in male inflorescences: their concentrations are similar to those found during early female flowering (De Keukeleire et al., 2003). The same authors demonstrated the presence of bitter acids and chalcones in leaves of fully grown hops even if their levels were found generally lower than in the hop cones and strictly related to the hop varieties (De Keukeleire et al., 2003, 2007). The hop leaves contain also volatile compounds but in a much lesser amount than the hop cones (<0.05%) (Langezaal, 1993).

The European Pharmacopoeia 5th ed. (2004) and the British Pharmacopoeia (2007) report the microscopical and chromatographical identification assays of hops (*Lupuli* flos). The thin-layer chromatogram of hop strobiles, examined in ultraviolet light at 254 nm, shows a number of quenching bands due to xanthohumol, humulones and lupulones.

6. Hop extracts

Immediately after harvesting, hop cones are carefully dried by artificial heat: water content must be reduced from 65–80% to 8–10% for storage. In the early nineteenth century extraction of hops was first attempted in water and ethanol (Gardner, 1987), but other methods have been also reported, such as the use of steam or carbon disulfide (Moir, 2000). The production of hop extracts has been improved in the last century, when the chemical structure and reactivity of the resin compounds have been elucidated. Owing to their lipophilic nature, a wide range of effective solvents, including alcohols, chloroform, acetone and hexane, has been used to dissolve the resin constituents. There was however a growing concern about the possible harmful effect of even small amounts of solvent residues in the extract. Therefore the technique of extraction by means of liquid or supercritical carbon dioxide was applied to hop cones. Carbon dioxide is a rather selective and non-polar solvent which is particularly suited to dissolve hop soft resin and oil, but it does not extract polar components or only traces of them. Some extracts obtained by supercritical carbon dioxide extraction of hop cones at four different combination of temperature (40–60 °C) and pressure (125–275 bar) were analyzed by means of HPLC and ^{1}H NMR spectroscopy (Langezaal et al., 1990). The authors observed that the extraction parameters influenced the yield and the composition of the mixture of the bitter compounds as well as the presence of volatile components. Among the different combinations of parameters tested, that of 40 °C and 200 bar was found the best condition for the extraction of both the bitter compounds and the volatiles.

It is important to underline that the knowledge of the active principles and the influence of the extraction procedure on extract composition can give clues to standardization and quality control.

7. Pharmacology

7.1. Sedative activity

The traditional use of hops as a mild sedative stems from the observation of sleepiness and fatigue in the hop-pickers, apparently due to the transfer of hop resin from their hands to their mouths (Tyler, 1987). The German Commission E approved hops for the treatment of "mood disturbances, such as restlessness and anxiety, sleep disturbances" (Blumenthal, 1998). Nevertheless the sedative activity of hops was poorly investigated in experimental and clinical studies. The first investigation carried out in rodents was published by Hänsel and Wagener (1967). The authors did not observe alteration in locomotor activity and in hexobarbital-induced sleeping time in mice orally treated with three types of hop extracts, two produced with ethanol and the third with methylisobutyl-ketone, at doses up to 500 mg/kg b.w. In addition neither antagonistic effect against metamphetamine-induced stimulation nor mus-

cle relaxation was found. The lack of a clear sedative effect was also reported in human subjects treated with 250 mg/day of a lipophilic hop extract for 5 days (Stocker, 1967). The tranquilizing property of different extracts of *Humulus lupulus*, intraperitoneally (i.p.) injected in mice, was investigated by Bravo et al. (1974). The authors observed a reduction in spontaneous motor activity, related to the type of solvent used in the extraction procedure. The ether extract was the most active in comparison with the aqueous and alcoholic ones. It must be underlined that a high dose, 1 ml of *Humulus lupulus* extract 10%/20 g b.w. was needed to elicit the reduction in motility. None of the tested extracts exerted a myorelaxant effect. The neuropharmacological effect of an undefined hop extract, dosed from 100 to 500 mg/kg, was evaluated in mice by Lee et al. (1993): hypothermic, analgesic and anticonvulsant activities were observed after i.p. injection. In addition sedative and hypnotic properties were ascribed to the hop extract following the observation of a dose-dependent reduction in spontaneous locomotor activity and a dose-dependent increase in pentobarbital-induced sleeping time.

The above-mentioned studies do not clearly demonstrate the sedative effect of *Humulus lupulus*. First of all the oral administration was applied only in the study of Hänsel and Wagener (1967) and they did not observe a sedative effect. The finding of this effect after the i.p. injection of hop extracts (Bravo et al., 1974; Lee et al., 1993) opens up a problem of bioavailability. Moreover the different extraction procedures and the undefined composition of the administered preparations make questionable the neuropharmacological activity of hops as well the identity of the active sedative principle/s. With regard to the last issue, Hänsel et al. (1980, 1982) attributed the sedative effect of hops to 2-methyl-3-butene-2-ol, deriving from hop constituents during storage at room temperature. This compound caused a 50% reduction of spontaneous motility without inducing a myorelaxant effect, when i.p. injected in rats at the dose of 206.5 mg/kg (Wohlfart et al., 1983a,b). A high dose (800 mg/kg) of the same compound was needed to induce narcosis in mice (Hänsel et al., 1980). It must be underlined that the hop extracts commercially available were found to contain small amounts of 2-methyl-3-butene-2-ol (<0.01%) (Hänsel et al., 1982), therefore it cannot be considered the major responsible constituent for the sedative effect of hop extract.

We recently investigated the neuropharmacological activity of *Humulus lupulus* using a CO_2 hop extract and single fractions containing α-acids and β-acids (Zanoli et al., 2005, 2007). CO_2 hop extract orally administered in rats exerted a pentobarbital sleep-enhancing effect in a dose-dependent manner, starting from a minimal effective dose of 10 mg/kg. The extract failed to affect the locomotor activity in the open field test and the anxious behaviour of rats submitted to the elevated plus-maze test. At our knowledge for the first time, we showed that hop extract, administered at the dose of 5–10 mg/kg b.w. three times (24, 5 and 1 h) before the test, reduced immobility time during the behavioural despair test, suggesting hence an antidepressant-like activity. The same pharmacological effects were elicited by the administration of hop fraction containing α-acids. On the other hand the fraction containing β-acids orally administered in rats (5–10 mg/kg) produced an increased exploratory activity in the open field, a reduction in the pentobarbital hypnotic activity and a worsening of picrotoxin induced seizures. In the elevated plus maze, the increased exploratory activity into the open arms showed by β-acid-treated rats, in comparison with controls, suggested a modest anxiolytic-like activity. In the forced swimming test, a significant reduction in the immobility time was observed in rats three-times treated with β-acids fraction (5 mg/kg b.w., 24, 5 and 1 h before the test). Electrophysiological studies performed on cerebellar granule cells in culture showed that the β-acids fraction decreased GABA-evoked current in a dose-dependent manner. In conclusion, α-acids fraction can be considered as the major responsible constituent for the enhanced pentobarbital effect and for the antidepressant property observed after the administration of CO_2 hop extract. The β-acids fraction exerted an antidepressant activity as well, but reduced pentobarbital hypnotic activity. In this context the behavioural (picrotoxin seizure) and electrophysiological results seem to suggest the ability of β-acids to reduce the GABAergic activity. The CO_2 extract and the two fractions of bitter acids share an antidepressant-like effect: this property could be particularly interesting taking in consideration the poor availability of medicinal plants useful for the treatment of depressive disorders.

A further study describing the sedative property of *Humulus lupulus* has been recently published by Schiller et al. (2006). The authors found a reduced locomotor activity, an increased ketamine-induced sleeping time and a reduced body temperature in mice treated with different dosages, from 200 to 500 mg/kg, of ethanolic and CO_2 hop extracts by oral gavage. These preparations were devoid of anxiolytic activity, thus confirming our previous results (Zanoli et al., 2005). In the same experimental conditions the authors tested also the effects of different fractions of hop extracts. Both fractions containing α-acids and β-acids were able to prolong ketamine-induced sleeping time, but the fraction containing β-acids needed a dosage approximately 6-times higher (200 mg/kg) than that of α-acids (25 mg/kg) in order to significantly potentiate the narcotic event. This last result seems to suggest a contribution of β-acids to the sedative activity of *Humulus lupulus*. The discrepancy between these results and our findings (Zanoli et al., 2007) should be elucidated taking in account several factors (raw material, storage condition, extraction procedure, type of solvent), besides the different applied dosages (Schiller et al., 2006).

A recent study showed that myrcenol, which is produced from myrcene during boiling hops, was able to prolong pentobarbital-induced sleeping time in mice and to potentiate GABAa receptor response *in vitro* (Aoshima et al., 2006). Taking in account the particular condition leading to the production of the tested compound, it is unlikely that myrcenol could play a role in the sedative effect of a hop extract. On the other hand myrcenol could represent a positive modulator of GABAa receptor response as a component of beer.

In spite of these recent studies, the identity of the active sedative principle/s of hops as well the mechanism/s of action is still questionable.

A study aimed to clarify the interaction of sedative herbs with selected central nervous system receptors demonstrated the capacity of a hop dried extract to bind serotoninergic 5-HT_6 receptors as well as melatoninergic ML_1 receptors (Abourashed et al., 2004). The involvement of 5-HT receptors in depression and sleep disturbances has been demonstrated (Shen et al., 1993) and the role of melatonin in the regulation of circadian rhythm is well-known (Pickering and Niles, 1990). It must be underlined that the tested extract contained 0.48% of flavonoids, but not bitter acids, owing to the utilization of a hydrophilic solvent in the extraction procedure (Abourashed et al., 2004). The involvement of melatoninergic system in the sedative effect of hops could be confirmed by the ability of luzindole, a melatonin receptor antagonist, to counteract the hypothermic effect of a hop methanolic extract (250 mg/kg) as well as that of melatonin (50 mg/kg) in BL6/C57J mice (Butterweck et al., 2007). In this study α-acids were excluded to be responsible for the hypothermic activity of hops because they were not present in the hydrophilic extract used in the experiments. This finding is not in accordance with those by other authors (Schiller et al., 2006) and by us (Zanoli et al., 2005), if the hypnotic event is really mediated by the hypothermic effect, as suggested by Gilbert et al. (1999).

An agonistic activity of hops at adenosine A1 receptors was excluded in a study aimed to investigate the mechanism of action of a valerian–hop combination dried extract (Müller et al., 2002). The authors suggested an alternative mechanism for the sedative effect of hops, probably involving GABA receptors (Müller et al., 2002). Both the authors of the reported studies (Müller et al., 2002; Abourashed et al., 2004) agree on the fact that *in vitro* activities need to be further substantiated by *in vivo* models.

The clinical investigations on the efficacy of hops in sleep disturbances were generally performed using preparations containing a combination of hops and other sedative herbs, particularly valerian. A randomized, double-blind, controlled trial in patients suffering from sleep disorders showed equivalent efficacy and tolerability between a hop–valerian preparation and a benzodiazepine drug (Schmitz and Jackel, 1998). Sleep quality was determined by psychometric tests, psychopathologic scales and sleep questionnaires. This study pointed out that the hop–valerian treatment for 2 weeks did not elicit the withdrawal symptoms, normally occurring with the benzodiazepine therapy.

The pharmacodynamic effects of a commercially available mixture of valerian and hops (Ze 91019) were studied in young adult patients using quantitative topographical electroencephalography (Vonderheid-Guth et al., 2000). A clear effect at the central nervous system level was observed 4 h after the intake of high dosage of the mixture (1500 mg valerian plus 360 mg hops).

A multicenter, randomized and placebo-controlled study was performed in 184 patients with mild insomnia, nightly administered for 28 days with a combination of standardized extracts of hops (83.8 mg) and valerian (374 mg) (Morin et al., 2005). Sleep parameters were measured by daily diaries and polysomnographic assays. The combination hops–valerian showed a modest hypnotic effect, improving sleep without producing significant residual effects and rebound insomnia. The lack of residual sedative effects was previously stressed by Gerhard et al. (1996) in healthy volunteers, receiving a hop–valerian combination or flunitrazepam, used as reference drug. The objective measurement of cognitive psychomotor performance and the subjective questionnaries on well-being led to emphasize the impairment of vigilance in the morning after the ingestion of the benzodiazepine drug, while more alertness and activity were observed in patients treated with the herbal remedy. Therefore the valerian–hop combination can be considered a useful and safe alternative to the classic sedative drugs (Gerhard et al., 1996; Schmitz and Jackel, 1998; Kubish et al., 2004; Morin et al., 2005). A herbal preparation, containing lavender oil, lemon balm and oat extracts besides hops, exhibited a relaxing effect, documented by electroencephalographic analysis, in healthy volunteers (Dimpfel et al., 2004). However the presence of valerian or other medicinal herbs in the clinical formulations does not allow to assess the potential clinical efficacy of hops administered alone.

7.2. Estrogenic activity

The frequent menstrual disturbances observed in female hop-pickers, during the early days of hop cones harvesting, suggested a potential hormonal activity of hops. In Germany, hop baths were traditionally used to treat gynaecological disorders. The presence of estrogenic substances in hops ("equivalent of 20–300 μg estradiol/g") was firstly suggested by Koch and Heim (1953). On the contrary other authors did not find estrogenic activity in hop essential oil, hop extracts, α-acids, β-acids and hop resin (Fenselau and Talalay, 1973). The discrepancy could be due to the different nature of extracts as well as to the variety of the specific assays used to determine estrogenic properties. In the study of Liu et al. (2001) the estrogenic activity of a methanol hop extract was demonstrated by: (a) the significant binding capacity to both estrogen receptors (ERα and ERβ); (b) the induction of alkaline phosphatase activity in Ishikawa cells (human endometrial adenocarcinoma epithelial cell line); (c) the up-regulation of progesterone receptor mRNA in Ishikawa cells; (d) the up-regulation of presenelin-2, an estrogen-inducible gene in S30 cells (breast cancer cell line transfected with ERα). These results were confirmed by Overk et al. (2005) using a chloroform partition of a methanol extract from a previously CO_2-extracted Nugget hops cultivar. The extract showed an estrogenic potency equivalent to that of a red clover (*Trifolium pratense* L.) ethanol extract: both demonstrated significant activities in the ER competitive binding, activation of transiently transfected ERE-luciferase, quantitative real-time PCR of an estrogen-inducible gene, and alkaline phosphatase enzyme induction assays.

Several phytochemical investigations were performed with the aim to identify the estrogenic principle, firstly named "hops-proestrogen" by Nastainczyk (1972) subsequently recognized as a mixture of 8-PN and 6-PN (Hänsel and Schulz, 1988). Among the different compounds (XH, IX, 6-PN, 8-PN) of a hop polyphenolic fraction showing estrogenic property, 8-PN displayed the major activity, measured *in vitro* using a sensitive bioassay based on the ability of estrogenic compounds to stimulate alkaline phosphatase activity in Ishikawa cells (Milligan

et al., 1999). In the same study, the high estrogenic potency of 8-PN was confirmed by its ability to interact with estrogen receptors in a radioligand binding assay on rat uterine cytosol. On the other hand 6-PN showed a very weak estrogenic activity (<1/100 of 8-PN) as isoxanthoumol did, while xanthoumol was inactive. These findings were subsequently confirmed in a yeast screen expressing the human estrogen receptor (Milligan et al., 2000). Using a mammalian cell-based transient transactivation assay, 8-PN was demonstrated to be approximately 100 times more potent than genistein, but unlike genistein, 8-PN displayed twofold higher affinity for ERα than ERβ measured by *in vitro* competitive binding assay (Schaefer et al., 2003).

The high estrogenic activity of 8-PN was also confirmed in different *in vivo* experiments. The subcutaneous administration of 8-PN (30 mg/kg/day) for 2 weeks was reported to suppress the decrease in bone mineral density and the reduction in uterine weight, induced in rats by ovariectomy (Miyamoto et al., 1998). 8-PN induced a characteristic estrogenic response in an acute *in vivo* test using uterine vascular permeability as an endpoint (Milligan et al., 2002) as well as in a 3-day uterotrophic assay in ovariectomized female rats (Diel et al., 2004). Recent studies performed *in vivo* demonstrated the capacity of 8-PN: (a) to reduce serum-luteinizing hormone (LH) and follicle-stimulating hormone (FSH); (b) to increase serum prolactin level and uterine weight; (c) to induce vaginal hyperplastic epithelium; (d) to cause secretion in the mammary glands of ovariectomized rats, after a 3-month treatment with a high dose (68.4 mg/kg) (Christofell et al., 2006; Rimoldi et al., 2006). These effects on the hypothalamo-pituitary-uterine axis are very similar (though milder) to the ones elicited by estradiol. A lower dose of 8-PN (18 mg/kg) daily administered in rats for 28 days was reported to prevent ovariectomy-induced trabecular bone loss (Hümpel et al., 2005). In these animals it was observed a minimal and dose-independent stimulatory effect on uterine cells; it was approximately 10-fold lesser than that of an equivalent bone protective dose of estradiol. This finding demonstrated a remarkable tissue specificity of 8-PN, which was confirmed in a transgenic reporter mouse model (Hümpel et al., 2005). The capacity of 8-PN to reduce menopausal hot flushes was recently assessed by Bowe et al. (2006), by measuring the tail skin temperature (TST) in ovariectomized rats. The subcutaneous daily administration of 400 μg/kg of 8-PN for 2 days resulted in a significant decrease in TST similar to that induced by estradiol (4 μg/kg). The effect of both substances was completely blocked by the peripheral estrogen receptor antagonist, ICI 182,780, thus demonstrating that peripheral mechanisms are involved

Table 1
In vitro biological activities of prenylflavonoids as potential cancer chemopreventive agents

Substrate/cell line	Biological activity	Main active components	IC_{50} values range (μM)	Reference
MCF-7 (human breast cancer cells), HT-29 (human colon cancer cells), A2780 (human ovarian cancer cells)	Antiproliferative activity	XH, DX, IX	0.5–15	Miranda et al. (1999)
PC-3, DU145 (human prostate cancer cells)		XH, DMX, IX, 6-PN, 8-PN	12–53	Delmulle et al. (2006, 2008)
HUVEC (human umbilical vascular endothelial cells)		XH	<10	Albini et al. (2006)
Mouse hepatoma Hepa 1c1c7 cells	Induction of quinone reductase (QR) activity	XH, 6-PN, 8-PN	1–10	Miranda et al. (2000a)
		XH, IX	7–35	Gerhaüser et al. (2002)
Isolated human LDL	Antioxidant and antiperoxidant	XH, DMX	5–25	Miranda et al. (2000b)
Rat liver microsomes		XH, DMX	5–25	Rodriguez et al. (2001)
CYP1A1, CYP1B1, CYP1A2, CYP3A4, CYP2E1	Inhibition of metabolic activation of procarcinogens	XH, IX, 8-PN	0.05–10	Henderson et al. (2000)
CYP1A2		XH, IX, 8-PN	2–10	Miranda et al. (2000c)
CYP1A		XH, IX, 6-PN, 8-PN	0.02–0.3	Gerhaüser et al. (2002)
Sheep seminal vesicle microsomes	Inhibition of cyclooxygenase enzymes:			Gerhaüser et al. (2002)
	COX1	XH, 8-PN	16–27	
	COX2	XH	41.5	
Mouse macrophage cells	Inhibition of nitric oxide synthase (iNos)	XH, IX	12–22	Gerhaüser et al. (2002)
		XH	1–5	Zhao et al. (2003)
Human placental vessels	Inhibition of angiogenesis	XH, IX	<10	Bertl et al. (2004)
Bovine endothelial cells		8-PN	3–10	Pepper et al. (2004)
Human colon cancer cells	Induction of apoptosis in tumor cells	XH	5–15	Pan et al. (2005)
MCF-7, T47-D (human breast cancer cells)		XH	<10	Vanhoecke et al. (2005a)
BPH-1 (human benign prostate hyperplasia cells), PC-3 (prostate cancer cells)		XH	10–20	Colgate et al. (2007)

XH, xanthohumol; DX, dehydrocycloxanthohumol; IX, isoxanthohumol; DMX, desmethylxanthohumol; 6-PN, 6-prenylnaringenin; 8-PN, 8-prenylnaringenin; CYP, cytochrome P450; LDL, low-density lipoprotein; IC_{50}, half-maximal inhibitory concentration.

in the regulation of the vasomotor response by estrogens and phytoestrogens.

In the study performed by Milligan et al. (2000) on the endocrine activity of hop flavonoids, none of the tested compounds (XH, IX, 6-PN, 8-PN) showed progestogenic or androgenic bioactivity. On the other hand, 8-PN was shown to possess anti-androgenic activity in a yeast-based androgen receptor assay (Zierau et al., 2003).

From the clinical point of view, a first randomized, double-blind, placebo-controlled study on the use of a standardized (on 8-PN) hop extract in menopausal women has recently been published by Heyerick et al. (2006). The daily administration of the extract, at a dose corresponding to 100 μg 8-PN for 6 weeks, to postmenopausal women decreased the incidence of hot flushes and other discomforts associated to estrogen deficiency (sweating, insomnia, heart palpitation, irritability). The efficacy of hop extracts in reducing hot flushes in menopausal women was previously suggested by Goetz (1990) and recently confirmed by the same author (Goetz, 2007) in a few number of patients treated with different types of non-standardized hop preparations. Vaginal dryness in postmenopausal women was significantly reduced by the topical application of a gel containing hyaluronic acid, liposomes, vitamin E and hop extract (Morali et al., 2006).

Single doses, from 50 to 750 mg, of 8-PN were orally given to healthy menopausal women in a randomized, double-blind, placebo-controlled study performed by Rad et al. (2006). The decrease in LH serum levels found after the highest dose demonstrated the ability of 8-PN to exert endocrine effects in menopausal women.

Although further clinical studies are needed, hop-derived prenylated flavonoids could provide an attractive alternative treatment for the relief of menopausal symptoms.

7.3. Cancer-related bioactivities

Over the past 10 years several *in vitro* studies have been carried out in order to evaluate the potential activity of hop components as chemopreventive agents. Some relevant data concerning the activity of prenylchalcones and bitter acids are presented in Tables 1 and 2, respectively. Among hop components, xanthohumol (XH) has received the major attention because it seems to inhibit *in vitro* initiation, promotion and progression stages of carcinogenesis, hence appearing as a broad-spectrum chemopreventive agent (Stevens and Page, 2004; Gerhaüser, 2005a; Colgate et al., 2007). A recent study performed *in vivo* showed the ability of XH to induce a significant inhibition of angiogenesis in mice implanted with a matrigel sponge, when administered in the drinking water at the concentration of 2 μM. At higher concentration (200 μM) XH displayed a marked angiogenesis inhibition without adverse effects on animal health parameters (Albini et al., 2006). In the same study the oral administration of XH at the concentration of 20 μM significantly inhibited the growth rate of KS-IMM tumors (Kaposi's sarcoma cell line) in male nude mice, starting from the 20th day of treatment. The inhibition of tumor angiogenesis and growth (33% and 83%, respectively, in comparison with controls) was observed by Gerhaüser (2005a) in female immuno-deficient mice implanted with human breast tumor xenograft and treated with XH sub-

Table 2
In vitro and *in vivo* biological activities of hop bitter acids as potential cancer chemopreventive agents

Substrate/cell line	*In vivo*/*in vitro*	Biological activity	Main active components	Concentration/dose	Reference
Mouse skin	*In vivo*	Inhibition of tumor promotion by TPA	H	1 mg/mouse topical application	Yasukawa et al. (1995)
Mouse bone cells	*In vitro*	Inhibition of bone resorption	H	5.9×10^{-9} M	Tobe et al. (1997a)
HL-60 (premyocytic leukaemia cell line), U937 (human monoblastic leukaemia)	*In vitro*	Induction of apoptosis	H	1–100 μg/ml	Tobe et al. (1997b)
HL-60	*In vitro*		α acids, β acids	50 μg/ml	Chen and Lin (2004)
SW620 (human metastatic colon carcinoma-derived cell line)	*In vitro*		L	40 μg/ml	Lamy et al. (2007)
U937	*In vitro*	Inhibition of cell proliferation	H	3.4 μM	Honma et al. (1998)
Endothelial cells	*In vitro*		H	10 μM	Shimamura et al. (2001)
HL-60, U937	*In vitro*		H	20–60 μg/ml	Chen and Lin (2004)
SW620	*In vitro*		L	10–60 μg/ml	Lamy et al. (2007)
Rat colon	*In vivo*	Inhibition of colon cancer induced by AOM	L	0.001–0.005% in drinking water	Lamy et al. (2007)
MC3T3-E1 (cells cloned from newborn mouse calvaria)	*In vitro*	Suppression of cyclooxygenase-2 gene transcription	H	1.6 μM	Yamamoto et al. (2000)
TPA-induced mouse skin tumor	*In vivo*		H	10 μmol/0.2 ml/mouse, topical application	Lee et al. (2007)
CAMs (chick embryo chorioallantoic membranes)	*In vivo*	Inhibition of angiogenesis	H	0.1–100 μg/CAM	Shimamura et al. (2001)

TPA, 12-*O*-tetradecanoylphorbol-13-acetate; H, humulone; L, lupulone; AOM, azoxymethane.

cutaneously injected at the dose of 1000 mg/kg b.w./day for 14 days.

7.4. Antibacterial and antifungal effects

Antibacterial activity, mainly towards Gram-positive bacteria, has been documented for hops and attributed to humulone and lupulone (Teuber and Schmalreck, 1973; Simpson and Smith, 1992; Oshugi et al., 1997). The activity of bitter acids towards Gram-positive bacteria, including some species of *Micrococcus*, *Staphylococcus*, *Mycobacterium* and *Streptomycetes*, has been thought to involve primary membrane leakage, due to the interaction of the hydrophobic parts of the molecules with the bacterial cell wall (Teuber and Schmalreck, 1973). The bitter acids were reported to exert antifungal activity against *Candida albicans*, *Trichophyton*, *Fusarium* and *Mucor* species. In particular humulones, exhibiting a minimal inhibitory concentration (MIC) of 100 μg/ml, were more active than lupulones (MIC > 200 μg/ml) against *Trichophyton* and *Mucor* spp., but less active against *Staphylococcus* spp. (MIC = 6.25 μg/ml vs. 3.13 μg/ml) (Mizobuchi and Sato, 1985). The authors investigated also the antifungal activity of prenylchalcones: XH and 6-PN were identified as the most potent agents against *Trychophyton* spp. (MIC = 3.13 μg/ml) and *Staphylococcus aureus* (MIC = 6.25 μg/ml) but they were practically inactive against other human pathogenic fungi (*Candida albicans* and *Fusarium* spp.) (Mizobuchi and Sato, 1984).

The essential oils obtained by hydrodistillation and chloroform extracts from different hop cultivars showed antimicrobial activity against Gram-positive bacteria (e.g. *Staphylococcus aureus*), but no influence on Gram-negative bacteria (e.g. *Escherichia coli*) and *Candida albicans* (Langezaal et al., 1992).

A recent review on the antiinfective properties of hop constituents, describes xanthohumol as a broad spectrum antiinfective agent against Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus mutans*), viruses (cytomegalovirus, herpes simplex virus type 1 and 2, human immunodeficiency virus 1), fungi (*Trichophyton* spp.) and malarial protozoa (*Plasmodium falciparum*) (Gerhaüser, 2005b). The mechanism/s of the observed inhibitory activities is/are still under investigation.

7.5. Stomachic effect

The traditional use of hops as a digestive herb was recently investigated in rats by Kurasawa et al. (2005). Using a pylorus-ligated model, the authors showed that a hop-dried extract, when orally administered, increased gastric juice volume without affecting acidity. The increased secretion was not observed after the intragastric administration of hops, suggesting that bitterness of hops is a crucial factor in inducing gastric secretion via the cephalic phase. The stomachic effect could be mediated by cholinergic nervous system since it was completely inhibited by atropine.

Clinically, the administration of an aqueous preparation of *Humulus lupulus* in patients affected by chronic hyposecretory gastritis showed a stimulating effect on gastric secretion (Torosyan and Mardzhanyan, 1974).

8. Side effects

Humulus lupulus can be responsible for allergic reactions in sensitive individuals. Pronounced signs of bronchial irritation, dry cough and dyspnea were observed in hop processing workers (Meznar and Kajba, 1990; Skórska et al., 2003). Respiratory impairment, together with immunological reactions (increased serum level of total IgE) was confirmed in brewery workers exposed to organic dusts such as hops, barley and brewery yeast (Godnic-Cvar et al., 1999). Contact dermatitis from hops was firstly described by Badham in 1834 (cited by Cookson and Lawton, 1953); subsequently several cases of occupational dermatitis to fresh and dried hops were reported by different authors (Cookson and Lawton, 1953; Raith and Jäger, 1984; Spiewak and Dutkiewicz, 2002). Nevertheless, at our knowledge no clinical case of allergy or anaphylaxis resulting from the therapeutic use of hops has been published.

Toxicological studies in animals stated that LD_{50} for orally administered hop extract in mice ranges from 500 to 3500 mg/kg (Hänsel et al., 1993). The oral administration of xanthohumol (5×10^{-4} M *ad libitum*) to laboratory mice for 4 weeks did not affect major organ functions and protein, lipid and carbohydrate metabolism (Vanhoecke et al., 2005b). Furthermore the subchronic oral administration of humulone derivatives in dogs was not associated with specific signs of toxicity demonstrating wide safety margins of these substances (Chappel et al., 1998).

9. Conclusions

In the last century and even more in recent years several researchers focused an increasing interest on *Humulus lupulus* and its components for their biological activities.

A traditional application of hops in humans consisted in the treatment of sleep disturbances: recent studies performed in rodents evidenced the sedative property of CO_2 hop extract and some of its fractions (Zanoli et al., 2005, 2007; Schiller et al., 2006). However different or even contradictory findings obtained by the authors concerning the activity of β-acids fraction require a detailed reinvestigation. Experimental evidence of hop sedating property has been produced in laboratory animals, but no randomized, double-blind, placebo-controlled clinical trial utilizing hop extract alone have been performed till now. No meaningful information regarding the potential clinical efficacy of hops can be extrapolated by using clinical formulations containing hops in combination with other medicinal plant, particularly valerian. Therefore the real efficacy of hops in sleep disturbances remains to be ascertained.

Today hop extracts are the major constituents of many food and dietary supplements with claim of "breast enhancement" (Coldham and Sauer, 2001) but also in this case properly controlled clinical trials supporting the use of hops for their estrogenic properties are still lacking. In addition no officially recognized standardization exists yet for estrogenic formulations of hops, even if the key compounds should be 8-PN for its estrogenic property, DMX as proestrogen and IX for its possible conversion *in vivo* to 8-PN. The experimental studies performed *in vitro* and *in vivo*, suggesting estrogenic properties for hop

extract or its single compounds, reasonably support the hypothesis that properly formulated hop preparations could represent an alternative to the classic hormone replacement therapy in the management of menopausal symptoms. However further research is required to assess the safety aspect, in particular the potential risk of breast or uterine cancer as a consequence of high-dosed supplements.

Hop research performed in the last decade has been largely dedicated to the biological activities of single hop components, particularly xanthohumol and isoxanthohumol among prenylflavonoids and humulone among bitter acids. Antiinflammatory, antioxidant, anti-lipoperoxidative activities as well antiangiogenetic, antiproliferative and apoptotic effects, mainly assessed in *in vitro* studies, reasonably suggest a potential chemopreventive activity. In addition these compounds proved to possess a broad-spectrum antiinfective activity against several microorganisms. However *in vivo* studies to assess their bioavailability, distribution, efficacy and safety in animal models are strongly recommended, before their application in humans.

The use of chemically characterized hop extracts for biological assays and for clinical trials is the right approach to study their pharmacokinetic and pharmacological profile and to perform comparative studies, with the aim to validate the abovementioned properties of hops. There is still a lot of work to be done in order to achieve a reliable standardized product and to link it to a specific biological activity and to specific therapeutic applications.

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