EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Assessment report on *Allium sativum* L., bulbus
Draft

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Allium sativum* L., bulbus
Herbal preparation(s)	- Powdered herbal substance - liquid extract from fresh bulb (DER 2-3:1), extraction solvent rapeseed oil, refined - Dry extract (DER 5:1), extraction solvent ethanol 34%
Pharmaceutical forms	Herbal preparation in solid dosage forms for oral use
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Peer reviewer	Ioanna Chinou

Note: This draft assessment report is published to support the public consultation of the draft European Union herbal monograph on *Allium sativum* L., bulbus. It is a working document, not yet edited, and shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on comments that will be received on this assessment report. The publication of this <u>draft</u> assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

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1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

The aim of this report is to assess the non-clinical and clinical data available on Allium sativum for preparing a European Union herbal monograph. This report is based on the documentation published in the literature.

- Herbal substance(s)

Allium sativum L., bulbus

The composition of Allium bulbus is complex. Garlic contains volatile oil (0.1-0.36%), the major components are sulphur compounds like alliin. It contains also proteins (amino acids, glutamyl peptides…), glucides, enzymes (alliinase, peroxidase, myronidase…) Allicin is formed from alliin by the alliinase. It is considered that 1 mg of alliin is equivalent to 0.45 mg of allicin. (Barnes *et al.,* 2002, ESCOP monograph, 2003, Paris *et al.,* 1981)

Relevant constituents of garlic

Organo-sulfur compounds, flavonoids, sapogenins and saponins, selenium compounds and fructosamines have been recognised as the main bioactive principles in raw garlic and different garlic supplements (i.e., garlic powder, garlic oil obtained either with steam distillation or maceration in vegetable oil, different aqueous/alcoholic extracts) (Berginc, 2012).

Organo-sulfur compounds notably alliin and allicin

Evidence from several investigations suggests that the biological and medical functions of garlic are mainly due to their high organo-sulphur compounds content (Omar *et al.,* 2010)

Flavonoids compounds

Apigenin, quercetin, nobiletin, tangeretin, rutin, allixin, myricetin and bergamottin from garlic are good antioxidants with potential cardio-preventive and antioxidants activities. However, their content in raw and processed garlic is very low (Lanzotti V, 2006). Consequently, their effects in the *in vivo* are expected to be negligible

Sapogenins and saponins

Garlic sapogenins and saponins (proto-eruboside B, eruboside B, proto-iso-eruboside B, iso-eruboside B, sativoside B1-5, R1, R2, β-chlorogenin and others) have been recently identified and their cholesterol-lowering effects in animals and *in vitro* antifungal, antitumor and cytotoxic activities have been confirmed (Lanzotti V, 2006)
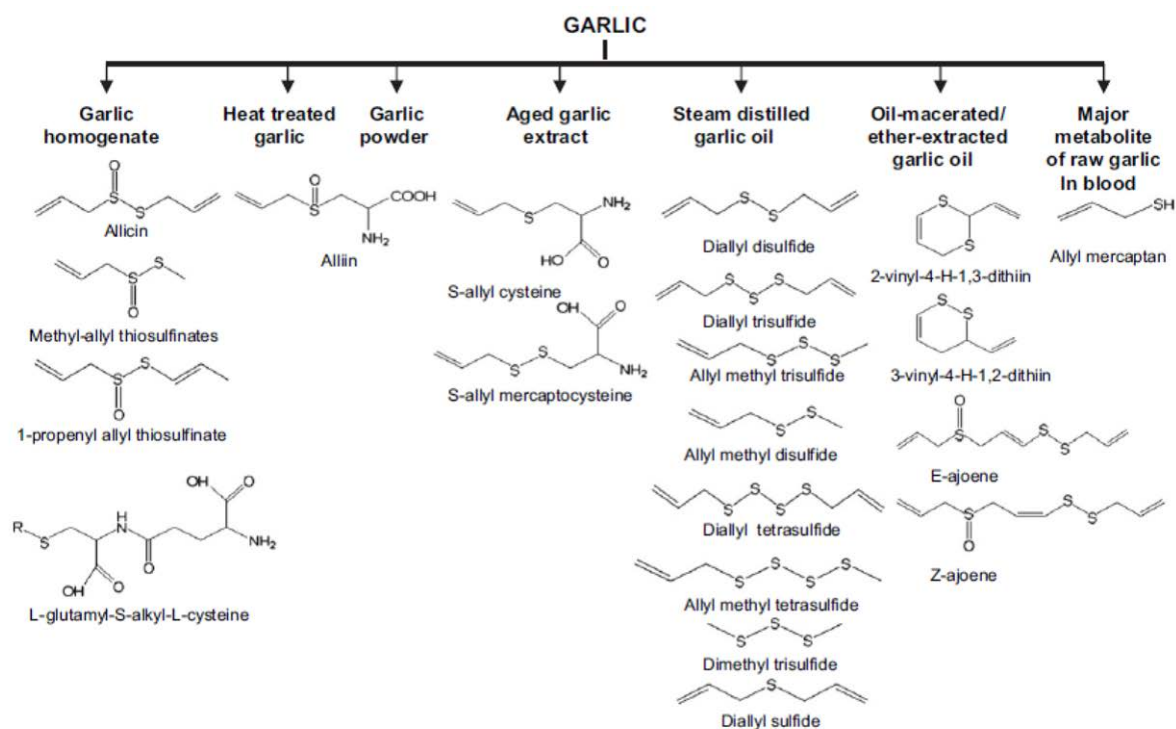
Fig. 3. Major organo-sulphur compounds present in different garlic preparations.

Figure 1: Major organo-sulphur compounds in garlic preparations

The European Pharmacopoeia prescribes no less than 0.45% of allicine (Eur. Ph., 2016)

• Herbal preparation(s)

Powdered herbal substance

Dry extract

• Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable

1.2. Search and assessment methodology

Online databases were used to research available non-clinical and clinical data on allium sativum preparations.

A research on the terms "Allium sativum", "Allium sativum bulbus", "Ail", "Allii sativi bulbus", "Garlic", "Pharmacology", "Side effect", "Adverse effect", "Undesirable effect", "Safety", "Drug Interaction", "Clinical trials", "Clinical studies", "Prevention", "Treatment", "Diabetes", "Metabolic diseases", "Glycaemia", "Antioxidant", "Dyslipemia", "Triglycerides", "Cholesterol LDL", "Lipoprotein", "Lipids", "Cholesterol HDL", "Hypercholesterolemia", "Atherosclerosis", "Cardiovascular disease", "Coagulation", "Platelet aggregation", "Peripheral arterial disease", "Blood Pressure", "Hypertension", "Medical claims", "Risk factors", "Antitumor", "Anti-tumorigenic", "Anti-proliferative", "Carcinogenesis", "Anti-carcinogenesis", "Chemoprevention", "Immunity", "Immunomodulation", "Fungal infections", "Anti-inflammatory activity", "Infection", "Antimicrobial" has been made the 14th February 2012, founding 422 references associated.

A pubmed research on garlic found 4147 articles in September 2013.

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Garlic bulb as single herbal substance is authorised in Denmark France, Germany, Hungary, Latvia, Poland, Spain, Sweden and United Kingdom.

The active substance is present on the market as:

- Herbal preparation

Powder (Denmark, 1987 and 2000; France, 1981; Germany, at least since 1976 (7 products, 1995, 1996 and 1999), Hungary, 1992 and 1994; Latvia, 1999; Spain, 1987 and 2006), United Kingdom, 1987 and 1990.

- Liquid extraction preparation from fresh bulb (DER 2-3:1), extraction solvent is rapeseed oil, refined (Germany, at least since 1976 and 2011)

- Dry extract (DER 5:1). Extraction solvent is ethanol 34% (Sweden, 1985)

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	Indication	Pharmaceutical form	Regulatory Status
Dried powder	For oral use. Herbal medicinal product in the prevention and treatment of mild hypercholesterolemia and hypertriglyceridemia as a supplement to a diet and where no other medical treatment is required	300mg corresponding to 3.5 mg alliin 1 tablet two times daily. Not to be used to children below 12 due to lack of experience	Since 2000 Denmark
Dried powder	For oral use. Herbal medicinal product in the prevention and treatment of mild hypercholesterolemia and hypertriglyceridemia as a supplement to a diet and where no other medical treatment is required	100 mg corresponding to 1.4 mg alliin.2 tablets 3 times daily	Since 1997 Denmark
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	200mg. 2 tablets 3 times daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	300mg. 1 tablet 3 times daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	210 mg. 2 tablets 3 times daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	300 mg. 1 tablet four times daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	250 mg. 5 tablets daily (2 after lunch, 3 after dinner)	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	230 mg. 2 tablets 3 times daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	250 mg. 5 tablets daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	330 mg. 1 soft capsule 3 to 4 times daily	1995 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	100 mg. 3 tablets 3 times daily	1996 Germany

Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	200mg. 2 tablets 3 times daily	1999 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalised arteriosclerosis	300 mg. 1 tablet 3 times daily	1999 Germany
Dried powder	For oral use 1) in adults: for the prevention of atherosclerosis, in cases of mild hypercholesterolemia, prevention of changes in blood vessels occurring with age 2) in adults and children over 3 years : Additional therapy in initial stages of upper respiratory infections	300 mg. 1) 1 to 2 tablets 2 to 3 times daily 2) adults: 2 tablets 3 to 4 times daily Children over 3 years: 1 tablet 2 times daily	1999 Latvia
Dried powder	traditionally used in the treatment of minor circulatory disorders	430 mg. 3 hard capsules daily	1981 France
Extract from fresh Allii sativi bulbus (2-3:1), extraction solvent Rapae oleum raffinatum	For oral use in adults: traditional used for prophylaxis of generalised arteriosclerosis	108 mg. 1 to 2 gastro-resistant capsules, soft 4 times daily	At least 1976 Germany
Extract from fresh Allii sativi bulbus (2-3:1), extraction solvent Rapae oleum raffinatum	For oral use in adults: traditional used for prophylaxis of generalised arteriosclerosis	108 mg. 1 to 2 gastro-resistant capsules, soft 4 times daily	2011 Germany
Dried garlic	For oral use in adults: for the prevention and complementary treatment of atherosclerosis, for the relief of cardiovascular disturbances deriving from atherosclerosis	100 mg corresponding to 1.3% amlliin, equivalent to 0.6 allicin. 1 dragee 3 times daily	1992 Hungary
Dried garlic	For oral use in adults: for the prevention and complementary treatment of atherosclerosis, for the relief of cardiovascular disturbances deriving from atherosclerosis	66 mg. 3 dragees 3 times daily	1994 Hungary
Dried powder	traditionally used for mild cardiovascular problems	330 mg. 1 to 2 hard capsules 2 times daily	1987 Spain

Dried powder	traditionally used for mild cardiovascular problems	400 mg. 1 hard capsule 3 times daily	2006 Spain
Dry extract, extraction solvent: ethanol 34%, DER : 5:1	For oral use in adolescents over 12 years and adults: traditionally used for alleviation of symptoms of common cold	100 mg corresponding to 500 mg fresh bulb. 1 to 2 tablets 1 to 2 times daily	1985 Sweden
Dried powder (+oil)	For oral use in adults and elderly: for the treatment of the symptoms of common cold and cough	300 mg (+ 0.001 ml). 3 tablets daily	1987 United Kingdom
Dried powder	For oral use in adults and children over 8 years : the relief of catarrh and rhinitis	150 mg. 3 tablets daily in adults and 2 tablets daily in children over 8 years.	1990 United Kingdom

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Table 2: overview of the marketed combination products

Active substance	Indication	Pharmaceutical form	Regulatory Status
Powdered garlic Powdered nettle	For oral use in adults: 1)in prophylaxis of upper respiratory infections 2) in prophylaxis of upper respiratory infections	200mg / 53.5 mg 1) 1 tablet 3 times daily. 2) 1 to 3 tablets 3 times daily	At least 1976 Poland

Information on other products marketed in the EU/EEA

Italy

Food supplements are on the market with indications: Regular cardiovascular system function. Triglycerides and cholesterol metabolism. Regular blood pressure. Fluidity of bronchial secretions. Nose and throat wellness. Digestive process. Antioxidant.

2.1.2. Information on products on the market outside the EU/EEA

Not applicable

2.2. Information on documented medicinal use and historical data from literature

Garlic has been used for thousands of years for culinary, medicinal and spiritual purposes. Garlic has been grown around the world, from Mediterranean climates to Siberia. Ancient Egyptians used it as a form of currency; its medical and magical powers were described on the walls of ancient temples and on papyrus dating to 1500 BC. Garlic cloves were buried in King Tut's tomb. Garlic was used by the Greek physicians, Hippocrates and Galen, and during the Middle Ages by Hildegard von Bingen. In the Middle Ages, garlic was used to ward off the evil eye, witches and vampires; it was also used as an aphrodisiac. In China, garlic was forbidden food for Buddhist monks because of its reputation as a sexual stimulant (Kemper K, 2000).

The name "Allium sativum" is derived from the Celtic word "all", meaning burning or stinging, and the Latin "sativum" meaning planted or cultivated. The English word, garlic, is derived from the Anglo-Saxon "gar-leac" or spear plant, referring to its flowering stalk (Kemper K, 2000), (Omar *et al.*, 2010)

Garlic has historically been used to treat earaches, leprosy, deafness, severe diarrhoea, constipation and parasitic infections, and to lower fever, fight infections and relieve stomach aches (Kemper K, 2000).

In Traditional Chinese Medicine, garlic is known as "da suan". It is considered a warm, bitter herb with particular effects on the Large Intestine, Spleen and Stomach meridians. It is used to lower blood pressure, to treat parasitic infections, food poisoning and tumours, and as a mild anticoagulant. It is traditionally contraindicated in patients with a yin deficiency (Kemper K, 2000).

Arabian herbalists use garlic to treat abdominal pain, infantile colic, diarrhoea, diabetes, eye infections, snake bites, dandruff and tuberculosis. African herbalists use garlic to treat respiratory infections and helminthic infections; many African families use garlic oil drops to treat childhood ear infections. In Ayurvedic medicine, garlic is used to treat respiratory problems, ulcers, colic and flatulence, and garlic oil drops are used to treat earaches. Several folk traditions recommend garlic as an emmenagogue or to induce abortions (Kemper K, 2000).In the 1800's, American physicians recommended garlic inhalation as a treatment for tuberculosis. Louis Pasteur demonstrated garlic's antiseptic activity in 1858, and Albert Schweitzer used it to treat dysentery in Africa. During World War I, garlic poultices were used topically to prevent wound infections in much the same way as described thousands of years earlier in the Talmud. By World War II, garlic had a reputation as "Russian penicillin" so prevalent was its use in a world in which antibiotics were in short supply (Kemper K, 2000).

American physicians relied on garlic as an antihypertensive agent up until the late 1950's. Although it was largely abandoned by mainstream physicians as more potent cardiovascular drugs and antibiotics became available, herbalists have continued to recommend it frequently (Kemper K, 2000).

Garlic is thought to have diaphoretic, expectorant, antispasmodic, antiseptic, bacteriostatic, antiviral, anthelmintic and hypotensive effects; it is commonly used to treat chronic bronchitis, recurrent upper respiratory tract infections and influenza. In Europe and India, garlic remedies are used to treat coughs, colds, hay fever and asthma. Many modern herbalists and folk healers still rely on garlic oil ear drops to heal the pain of a child's ear infection (Kemper K, 2000).

Allium sativum is probably indigenous to Asia but it is commercially cultivated in most countries (WHO) and is clearly one of the most popular herbal remedies worldwide today.

Steinegger & Hänsel, 1972: *Allium sativum* has been used by Egyptian, Indian, Phoenician, Roman, Griek and German. It has been used for chronic bronchitis, asrhma, and woopping cough.

Madaus, 1979: *Allium sativum* has been used for centuries for many therapeutic area asthma, scurvy, pinworm, cholera, thyphus, chronic bronchitis, diphtheria, influenza, antiseptic, expectorant, diaphoretic, arteriosclerosis...

For example, the following dosages are given:

Fresh bulb: 2-8 g
Infusion: 30 g
Juice: 20 g
Tincture: 20-50 drops

Based on the feedback obtained from Member States, a use is reported for a long period in the EU.

The WHO-Monograph states uses supported by clinical data as an adjuvant to dietetic management of hyperlipidaemia, and the prevention of atherosclerotic (age-dependent) vascular changes and mild hypertension. It also states uses described in pharmacopoeias and in traditional systems of medicine for the treatment of respiratory and urinary tract infections, ringworm and rheumatic conditions. The herb has been used as a carminative in the treatment of dyspepsia. Many uses are also described in the literature but not supported by experimental or clinical data, as an aphrodisiac, antipyretic, diuretic, emmenagogue, expectorant, sedative, to treat asthma and bronchitis and to promote hair growth.

Available dosage recommendations are the following:

Unless otherwise prescribed, average daily dose is as follow:

fresh garlic: 2-5 g
dried powder: 0.4-1.2 g
oil: 2-5 mg
extract: 300-1000 mg (as solid material).

Other preparations should correspond to 4-12 mg of alliin or about 2-5 mg of allicin

Bulbus Allii Sativi should be taken with food to prevent gastrointestinal upset.

According to ESCOP monograph, *Allium sativum* is indicated for prophylaxis of atherosclerosis and for the treatment of elevated blood lipid levels insufficiently influenced by diet. It is also used for upper respiratory tract infections and catarrhal conditions although clinical data to support this indication is not available.

Available dosage recommendations are the following:

Prophylaxis of atherosclerosis or treatment of elevated blood lipid levels

Adults: The equivalent of 6-10 mg of alliin (approx. 3-5 mg of allicin) daily, typically contained in one clove of garlic or in 0.5-1.0 g of dried garlic powder.

Upper respiratory tract infections

Adults: 2-4 g of dried bulb or 2-4 ml of tincture (1:5, 45% ethanol) three times daily.

British herbal Pharmacopoeia, 1976 and 1983: *Allium sativum* is indicated for chronic bronchititis, respiratory catarrh, recurrent colds, whooping cough, bronchitic asthma and influenza.

The preparations can be used in children but only small doses diluted should be administred.

Martindale, 1972: *Allium sativum* is indicated as expectorant, diaphoretic disinfectant and diuretic. The syrup has been used in the treatment of chronic bronchitis and other pulmonary conditions. The preparations of the garlic juice end the garlic syrup were given in the Britishs pharmacopeia codex (1949)

Table 3: Overview of historical data

Herbal preparation	Documented Use/ Traditional Use	Pharmaceutical form	Reference
a) fresh garlic b) dried powder oil extract e) Other preparations	To prevent gastro-intestinal upset	2-4 g 0.03-0.12 ml	1999 WHO monograph
a) Clove b) Powder c) Dried bulb d) Tincture (1:5, ethanol 45%)	a) et b) prophylaxys of atherosclerosisor treatment of elevated blood lipid level b) et d) Upper respiratory tract infections	a) 6-10 mg of alliin (3-5 mg of allicin) b) 0.5-1.0 g c) 2-4 g d) 2-4 ml	2003 ESCOP
a) Dried bulb b) Tincture c) Syrup d) Oil	Chronic bronchitis, respiratory catarrh, recurrent colds, whooping cough, brochititic asthma, influenza.	a) 2-4 g b) 2-4 ml c) 2-4 ml d) 0.03-0.12 ml	1976, 1983 British herbal pharmacopoeia
a) Fresh garlic b) Garlic juice c) Garlic syrup	Expectorant, diaphoretic disinfectant and diuretic. The syrup has been used in the treatment of chronic bronchitis and other pulmonary conditions	a) 2-8 g b) 2-4 ml c) 2-8 ml	1972 Martindale The extra Pharmacopoeia

2.3. Overall conclusions on medicinal use

According to the market overview the following garlic preparations fulfil the criteria to be eligible for the traditional use.

Table 4: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
Dried powder. Hard capsules	traditionally used in the treatment of minor circulatory disorders	430 mg. 3 capsules daily	1981 France
Dried powder. Tablets	prophylaxis of generalised arteriosclerosis	200mg. 2 tablets 3 times daily	At least since 1976 Germany
Dried powder. Tablets	prophylaxis of generalised arteriosclerosis	300mg. 1 tablet 3 times daily	At least since 1976 Germany
Dried powder. Tablets	prophylaxis of generalised arteriosclerosis	210 mg. 2 tablets 3 times daily	At least since 1976 Germany

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
Dried powder. Tablets	prophylaxis of generalised arteriosclerosis	300 mg. 1 tablet four times daily	At least since 1976 Germany
Dried powder. Tablets	prophylaxis of generalised arteriosclerosis	250 mg. 5 tablets daily (2 after lunch, 3 after dinner)	At least since 1976 Germany
Dried powder. Tablets	prophylaxis of generalised arteriosclerosis	230 mg. 2 tablets 3 times daily	At least since 1976 Germany
Dried powder. Tablets	prophylaxis of generalised arteriosclerosis	250 mg. 5 tablets daily	At least since 1976 Germany
Extract from fresh Allii sativi bulbus (2-3:1), extraction solvent Rapae oleum raffinatum. Gastro-resistant capsules, soft	traditional used for prophylaxis of generalised arteriosclerosis	108 mg. 1 to 2 capsules 4 times daily	At least 1976 Germany
Dry extract, extraction solvent: ethanol 34%, DER : 5:1. Tablets	traditionally used for alleviation of symptoms of common cold	100 mg corresponding to 500 mg fresh bulb. 1 to 2 tablets 1 to 2 times daily	1985 Sweden

According to the data from the literature, some preparations are quoted in books for more than 30 years (British pharmacopeia, Martindale), but they were used for many therapeutic indications with the same posology. Most indications are not suitable for traditional use (chronic bronchitis, asthma, whooping cough…).

3. Non-Clinical Data

Non-clinical strategy

Online databases were used to research available non-clinical data on garlic preparations (extracts, oil and its relevant constituents). No data was provided by the EMA on behalf of interested parties.

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

The WHO described the medicinal uses of garlic, either supported by clinical data, as an adjuvant to dietetic management in the treatment of hyperlipidaemia and in the prevention of atherosclerotic vascular changes. The plant may be useful in the treatment of mild hypertension (WHO, 1999). The ESCOP monograph (2003) reported also anti-aggregatory and antioxidant effects. The effects are primarily due to allicin and its transformation products.

The literature reported large number of references of non clinical data for garlic and its constituents. Some controversial results are reported in non clinical studies, one of possible explanations for the discrepancy may result from the different constituents of garlic or garlic preparations used and different durations and design study.

Garlic effects observed in animal studies have been largely studied in human studies (see section 4 – Clinical Data).

In the review, only some non clinical references are mentioned in order to support the major garlic effects.

3.1.1. Primary pharmacodynamics

Lipid-lowering effects

Garlic preparations were shown to have lowering effects on cholesterol and plasma lipids, lipid metabolism and atherogenesis both *in vitro* and *in vivo*.

In vitro

Primary hepatocyte cultures treated with a high concentration of garlic extracts showed anticholesterogenic properties (Yeh *et al.,* 1994).

Garlic powder diminished cholesterol biosynthesis in cultured rat hepatocytes with an IC50-value is 90 micrograms/ml and human HepG2 cells with an IC50 value of 35 micrograms/ml (Gebhardt, 1993).

In vivo

Anti-hypercholesterolaemic and anti-hyperlipidaemic effects were observed in various model (rat, rabbit, chicken, pig) after oral (in feed) or intragastric administration of fresh garlic, garlic extract and garlic oil (Ismail *et al.,* 1999). Oral administration of garlic powder (50 mg/kg) to hypercholesterolemic rats during 6 weeks period reduced blood cholesterol and triglycerides levels (Ali and al, 2000).

Garlic extract has also been shown to decrease development of fatty streak and fibro fatty plaques in rabbits and mouse (Abramovitz *et al.,* 1999 and Liu *et al.,* 2001).

The mechanism of lipid-lowering effects appears to be in interaction at the molecular level with the phosphorylation cascade of hydroxymethyl-glutaryl-CoA reductase and garlic constituents (Gebhardt *et al.,* 1994).

In addition, during a 24-hour incubation period, garlic powder significantly reduced the level of cholesteryl esters by 26% and free cholesterol by 32% in cells of atherosclerotic plaques of human aorta ($p<0.05$) and inhibited their proliferative activity by 55% at 1 mg/ml (Orekhov *et al.,* 1995).

Antihypertensive effect and effect on vascular resistance

The antihypertensive activity of garlic has been demonstrated *in vivo*.

Fresh garlic extracts have been found to lower blood pressure in spontaneously hypertensive rats and in anesthetised dogs where garlic extract was introduced via a femoral cannula and hypotensive effect has also been described in various normotensive experimental animals (Al-Qattan, 1999).

An aqueous garlic extract administered orally as a single or as repeated doses at 50 mg/kg body weight for 2 weeks in rat two-kidney-one-clip Goldblatt model (hypertensive rats) exerted a significant antihypertensive effect (Al-Qattan, 1999).

In addition, systolic blood pressure was significantly decreased after garlic powder administration (0.5% in diet) in hypertensive rats (Brandle *et al.,* 1997).

Garlic extracts elicited a marked vasorelaxant effect, which depends, in part, on the synthesis and release of NO (Das *et al.,* 1995 and Fallon *et al.,* 1998) and in other part, on the of endothelin-1 contraction (Kim-Park, 2000). Fresh garlic appears to change the physical state functions of the membrane potential. The potassium channels opened frequently causing hyperpolarisation which result in vasodilatation because the calcium channels were closed (Siegel *et al.,* 1992).

Effect on platelet aggregation

A number of studies have suggested the possible use of garlic as an antithrombotic agent.

An aqueous garlic extract inhibited collagen-stimulated platelet aggregation in platelet-rich plasma with IC50 of 460µg/ml (Lawson *et al.*, 1992).

Garlic extracts inhibited platelet aggregation *in vitro* via the ADP pathway and to lesser extent aggregation induced by epinephrine (Hiyasat *et al.*, 2009).

It has been reported that garlic and various garlic preparations reduce platelet preparation and production of thromboxane B2 *in vivo* in rats. The serum thromboxane B2 levels of the animals treated were measured as an index of the efficacy of antithrombotic agent, the thromboxane B2 was significantly inhibited after 50 mg/kg of aqueous extract of garlic orally and intraperitoneally in rats (Bordia *et al.*, 1996).

Hypoglycaemic effect

Hypoglycaemic effects of garlic preparations and its constituents have been demonstrated in diabetic rats. After oral administration of 200 mg/kg body weight of garlic bulb in Streptozotocin-nicotinamide diabetic rats, a significantly decrease of hyperglycaemia have been reported by increasing the production of insulin (26-37%) (Madkor *et al.*, 2011). However, similar studies reported negative results; garlic bulbs administrated orally (in diet, 6.25% by weight) to normal or streptozotocin diabetes mice had no effect on hyperglycaemia or hypoinsulinaemia (Swanston-Flatt *et al.*, 1990).

Antioxydant effect

Garlic preparations prevented tumour promotion (Balasenthil *et al.*, 1999; Lamm *et al.*, 2000; Tsubura *et al.*, 2011), cardiovascular diseases (Prasad, 1997), liver damage (Obioha, 2009), kidney damage (Kabasakal, 2005) and aging (Brunetti, 2009), which are considered to be associated with oxygen radical and lipid peroxidation The intrinsic antioxidant activity of garlic and some of its constituents have been well documented *in vivo* and *in vitro* (Kabasakal *et al.*, 2005).

The aqueous extract obtained from 1 mg of the garlic preparation was found to be anti-oxidatively as effective as 30 nmol of ascorbic acid and/or 3.6 nmol of alpha-tocopherol by photochemiluminescence method (Popov *et al.*, 1994).

The radical scavenging properties of garlic preparations against oxygen radicals, specifically their ability to inhibit the formation of superoxide anions, were investigated using human granulocytes activated with 10 nM phorbol myristyl acetate (PMA). A garlic powder preparation inhibited the production of superoxide with a calculated IC50 of 390 micrograms/ml. Sulfur containing constituents of garlic are considered responsible for conveying the antioxidative properties of garlic preparations (Siegers *et al.*, 1999).

3.1.2. Secondary pharmacodynamics

The WHO and ESCOP described a large of antibacterial and antifungal activity: garlic preparations inhibit the *in vitro* growth of *Bacillus species, Staphylococcus aureus, Shigella sonnei, Erwinia carotovora, Mycobacterium tuberculosis, Escherichia coli, Pasteurella multocida, Proteus species, Streptococcus faecalis, Pseudomonas aeruginosa, Candida species, Cryptococcus species, Rhodotorula rubra, Toruloposis species, Trichosporon pullulans, and Aspergillus niger*. These effects have been well documented in many references in literature.

3.1.3. Safety pharmacology

No study available

3.1.4. Pharmacodynamic interactions

No study available

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

No pharmacokinetics data about garlic preparations were available.

The metabolism of different garlic constituents was investigated using the experimental model of the isolated perfused rat liver (Egen-Schwind, 1992). Allicin showed a remarkable first pass effect and passed the liver unmetabolised only at high concentrations. Diallyl disulfide and allyl mercaptan were identified as metabolites of allicin, whereby diallyl disulfide probably is the metabolic precursor of allyl mercaptan.

The pharmacokinetic properties of S-allycysteine were studied after orally administration in rat, mouse and dog. This garlic constituent is a water-soluble transformation product from garlic. S-allycysteine was rapidly and easily absorbed, distributed mainly in plasma, liver and kidney; the bioavailability was 98.2% in rats, 103% in mice and 87.2% in dogs and was mainly excreted into urine (Nagae *et al.*, 1994).

Pharmacokinetic interactions

One study of on Phase I and Phase II biotransformation enzymes was reported in literature (Davi, 1992). Rats treated with a single dose of garlic oil (500 mg/kg i.p.) showed a significant dose-dependent depression of hepatic cytochrome P-450.

An *in vitro* study was performed to ascertain the risk potential for generating interactions with therapeutic products. Extracts of fresh garlic and garlic preparations were tested using the major cDNA-expressed human cytochrome P-450 isozymes associated with the metabolism of HIV/AIDS drugs, and purified P-glycoprotein (P-gp) cell membranes. Extracts of fresh garlic, garlic oil and freeze dried garlic exhibited an inhibitory effect on cytochrome P450 2C9*1, 2C19, 3A4, 3A5 and 3A7 mediated metabolism of a marker substrate. The activity of 2D6 mediated-metabolism was generally unaffected by garlic. Extracts of the fresh garlic stimulated CYP2C9*2 metabolism of the marker substrate. With the extracts tested, garlic had very low to moderate P-gp interaction as compared with the positive control verapamil. The findings demonstrate that garlic components can affect cytochrome P-450 2C, 2D and 3A mediated-metabolism of the isoforms studied. The safety and efficacy of conventional therapeutic products metabolised by the affected isozymes, particularly those with a narrow therapeutic index, taken concomitantly with garlic needs to be examined further under clinical settings (Foster *et al.*, 2001).

An *in vitro* study was performed in immortalised human hepatocytes (Fa2N-4 cells). Exposure of hepatocytes to garlic extract (0-200 µg/ml) may reduce the expression and activity of CYP2C9 with no detectible effects on CYP3A4 (HO *et al.*, 2010).

Drug interaction: fresh garlic extract and propranolol

A study was conducted in rats to assess the pharmacokinetic interaction between propranolol (10mg/kg po) and fresh garlic extract (250 mg/kg po). The coadministration led to an increase of propranolol Cmax and a prolonged elimination half-life (from 2.2 to 6.6h). The propranolol clearance

was also reduced (from 5.26 ml/kg/h to 1.72), the rate of absorption and elimination was reduced. Garlic in this study increases the bioavailability and decreases the elimination of propranolol (aq 2010).

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

3.3.1. Single dose toxicity

The data on acute toxicity of garlic preparations and its constituents were found in many references and in ESCOP and WHO monographs.

Garlic is considered to have very low toxicity and is listed as Generally Recognised as Safe (GRAS) by the U.S. Food and Drug Administration.

Different references are mentioned in the report of evaluation of health aspects of garlic and oil of garlic as food ingredient which was written by FDA in February 1973.

The available data are summarised in Table 5.

Table 5: summary of single dose toxicity studies

Ref.	Formulation	Species	Route, dose	Noteworthy findings
Garlic Preparations				
Torrescasana, 1946 (cited in FDA evaluation for the GRAS status, 1973)	Partially purified aqueous alcohol extract of garlic bulbs	Rat/Mouse	Intravenous	LD50 not shown. Authors reported the "LD50" as equivalent of garlic unit per kg, "LD50" equivalent of 500 g of garlic per kg
Nakagawa *et al.*, 1984	Garlic extract (type of extract not specified)	Wistar Rat ddY mouse	Per os Intraperitoneal Subcutaneously	LD50 > 30 ml/kg
Mills *et al.*, 2005	Aqueous extract of crushed fresh garlic	Rat	Undefined	LD50 = 173.8 ml/kg
Nwanjo *et al.*, 2007	Aqueous garlic extract	Rat (6 rats/group)	Intraperitoneal 0, 100, 200, 400, 800 and 1600 mg/kg	LD50 was calculated by the method of Litchfield and Wilcoxon (1949) LD50 = 625.08 mg/kg
Mikail *et al.*, 2010	Bulb aqueous extract	New Zealand rabbit (3 animals per group)	Subcutaneously 0, 300, 600, 1200, 2200, 3200, 4200 mg/kg	LD50 was determined using the Arithmetic method of Kraber modified by Aliyu and Nwude (1982) LD50 = 3034 mg/kg Maximum Tolerated Dose (MTD) = 2200 mg/kg **Post mortem examinations:** 300, 600, 1200, 2200 mg/kg: no discernible gross pathological lesion, no death

Detail of findings for Mikail *et al.*, 2010:

Dose (mg/kg)	Route	Toxicity sign	Mortality
300	S.C	No toxic signs noticed	No death recorded
600	S.C	No toxic signs noticed	No death recorded
1200	S.C	No toxic signs noticed	No death recorded
2200	S.C	Became dull after 4 h, loss of appetite, became active and regain appetite in 12 h time	No death recorded
3200	S.C	Became dull in 3 h time with glossy eyes, eyes closed, layed down for about 3 h, loss of appetite, partial paralysis of the fore limbs.	Two died after 48 and 72 h
4200	S.C	Became dull within 3 h time with glossy eyes, eyes closed, layed down for about 3 h, loss of appetite, partial paralysis of the fore limbs.	All died between 48 to 72 h.
Distilled water	S.C	No toxicity	No death

Ref.	Formulation	Species	Route, dose	Noteworthy findings
				3200 and 4200 mg/kg: slightly congested liver with recorded numbers of death.
Joseph *et al.*, 1989	Garlic oil	Male albino rat	100 mg/kg body weight Intragastrically	All died (6/6) - cause of death appears to be acute pulmonary oedema - all organs revealed severe congestion on the histopathological examination
Garlic constituents				
Raghunandana, 1949 (cited in FDA evaluation for the GRAS status, 1973)	Allicin	Mouse	Subcutaneous	LD50 = 50 mg/kg
Raghunandana, 1949 (cited in FDA evaluation for the GRAS status, 1973)	Allicin	Mouse	Intraperitoneal	LD50 = 20 mg/kg
Cavallito, 1944 (cited in ESCOP, 2003)	Allicin	Mouse	Intravenous	LD50 = 60 mg/kg
Cavallito, 1944 (cited in ESCOP, 2003)	Allicin	Mouse	Subcutaneous	LD50 = 120 mg/kg
Mills *et al.*, 2005	Allicin (as an oil)	Mouse	Undefined	LD50 = 0.2 ml/kg
Christensen, 1972 (cited in FDA evaluation for the GRAS status, 1973)	Diallyl sulfide	Mouse	Intraperitoneal	LD50 = 500 mg/kg

Assessor's comment

No data were found in the literature on the acute toxicity on the garlic powder.

Study performed by Joseph et al.,(1989) suggests a warning with garlic oil but the studies performed with the garlic extracts are reassuring, including the study performed by Mikail et al.,(2010) with a unusual model by an unusual route (LD50 = 3034 mg/kg).

3.3.2. Repeat dose toxicity

The toxic effects were studied and reported in references from 1939 to 2009. Some references are reported in FDA evaluation for the GRAS Status and in the ESCOP monograph. Garlic powder, fresh garlic, garlic juice and garlic extracts were evaluated in rat and guinea pig.

The available data are summarised in Table 5.

Two old studies on garlic powder were reported in report of evaluation of health aspects of garlic and oil of garlic as food ingredient which was written by FDA in February 1973. The authors reported that in one of these studies "it was noted on a diet of 5% dehydrated garlic, the second generation were sterile".

Two more recent studies reported inhibitory effects on male reproductive functions with garlic.

Dixit and Joshi (1982) treated male rats with garlic powder (50 mg garlic powder for 45 days or 70 days by oral route). After oral administration of 50 mg of garlic powder for 45 days, the testes showed degenerative changes but in most of the tubules normal stages from spermatogonia to spermatids have been seen; seminiferous tubule and Leydig cells nuclei were shrunken. After oral administration of 50 mg of garlic powder for 70 days, severe testicular lesions were seen. Spermatogenesis was arrested at the primary spermatocyte stage; Sertoli cells also showed degenerative changes.

Hammami *et al.,* (2008) treated male rats with crude garlic for one month (5, 10, 15 and 30% in diet). A significant decrease was observed in the body weight (at 15 and 30%), the prostate weight (at 30%) and of seminal vesicle weight (at 10, 15 and 30%). In contrast, testis and epididymis weights were unchanged. In epididymis tissue, the alpha glucosidase activity and the spermatozoa density were unchanged. The treatment resulted in a significant decrease in testosterone serum levels (at 10, 15 and 30%) associated with a significant increase in LH serum levels. Testicular histology showed a dose-dependent increase in the percentage of empty seminiferous tubules. Moreover, testicular function was affected; a significant decrease in phosphatase acid activity and testosterone contents were observed. The authors conclude that test-article reduces testosterone secretion and alters spermatogenesis at 10%, 15% and 30% doses and the authors specifies that their results are in accordance with the study by Dixit and Joshi (1982).

The authors show that crude garlic feeding altered the reproductive male function in adult male rats inaccessory glands (prostate, epididymis and seminal vesicle) and testis (spermatogenesis). This action is probably related to an effect of garlic on the Leydig cells, and perhaps also on the Sertoli cells. Hammami *et al.,* (2009) show that feeding with crude garlic inhibited Leydig steroidogenic enzyme expression and Sertoli cell markers. These alterations might induce germ cell death (spermatocytes and spermatids) via an apoptotic process.

Table 6: Summary of repeat-dose toxicity studies

Ref.	Formulation	Species	Duration, route, dose	Noteworthy findings
Rodent studies				
Jubb, 1947 (cited in FDA evaluation for the GRAS status, 1973)	Garlic powder	Wistar rat	- duration not known - oral route 2.5% dehydrated garlic (equivalent to 10% fresh garlic)	*(endpoint not detailed)* ↓ Hb ↓ RBC
Jubb, 1947 (cited in FDA evaluation for the GRAS status, 1973	Garlic powder	Wistar rat	- duration not known - oral route 5% dehydrated garlic (equivalent to 20% fresh garlic)	*(endpoint not detailed)* The second generation rats were sterile
Dixit and Joshi, 1982	Garlic powder	White albino rats	- Group I : vehicle - Group II : 50 mg garlic powder each day (45 days) - Group III : 50 mg garlic powder each day (70 days)	*(endpoints: body and testes, epididymis, seminal vesicles, adrenal, liver weights, seminiferous tubular and Leydig cells nuclear diameters, protein, sialic acid and cholesterol contents of testes, epididymis, seminal vesicle, liver and heart muscle, blood sugar and serum analysis, histology on right testis)* - ↓ bodyweight - ↓ testes, epididymis and seminal vesicle weight - ↓ protein, sialic content of testes, epididymis and seminal vesicles - ↓ hepatic and cardiac muscle cholesterol - ↓ blood sugar, serum protein, cholesterol, phospholipid, triglyceride and SGPT After 45 days of treatment : degenerative changes in testes (seminiferous tubule and leydig cells nuclei were shrunken) but normal stages from spermatogonia to spermatids After 70 days of treatment : - Severe testicular lesions - Spermatogenesis arrest at the primary spermatocyte stage - Sertoli cells degenerative changes

Ref.	Formulation	Species	Duration, route, dose	Noteworthy findings
Carl, 1939 (cited in FDA evaluation for the GRAS status, 1973)	Fresh garlic	Rat (5)	- duration not known - oral route 20 to 30% in diet	*(endpoint not detailed)* All animals died within 11 days
Hammami *et al.*, 2008	Crude garlic	Wistar rat	- 30 days - oral route - 5, 10, 15 and 30% in diet - 30 rats	*(endpoints: body and reproductive organ weight, tissue biochemistry on testis, epididymis and prostate and seminal vesicle, sperm density, histology, statistical analysis)* Body and organ weights - ↓ bodyweight - ↓ seminal vesicle and prostate weight Hormonal measurements - ↓ serum testosterone level with ↑ LH concentration Biochemistry on accessory gland - ↓ prostate citric acid - ↓ seminal vesicles fructose Testicular analysis : - morphological alterations of seminiferous tubules - ↑ percentage of empty seminiferous tubules - cellular alterations in testicular ultrastructure - nuclear degeneration in the primary spermatocytes ans spermatids - Sertoli cells : reduced volume, vacuolization, sparse organelles, few scattered mitochondria - Leydig cells : more lipid droplets - ↓ acid phosphatise activity - ↓ intra-testicular testosterone concentration
Hammami *et al.*, 2009	Crude garlic	Wistar rat	- 30 days - oral route - 5, 10, 15% in diet - 24 rats	*(endpoints: testicular markers)* - ↑ expression of active Caspase 3 - ↑ expression of caspase inhibitors BIRC3 and BIRC2 - ↑ expression of mitochondrial pro-apoptotic factor IAP inhibitor DIABLO - ↓ expression of steroidogenic enzymes (Star, Cypl la, HSD3b5 and Hsd17b)

Ref.	Formulation	Species	Duration, route, dose	Noteworthy findings
Torrescasana, 1946(cited in FDA evaluation for the GRAS status, 1973)	Partially purified aqueous alcohol extract of garlic bulbs	Rat	- duration not known - oral route Authors reported the dose as equivalent of garlic unit per kg, equivalent of 138 g of garlic per kg	*(endpoint not detailed)* ↓ loss of weight
Joseph *et al.*, 1989	Aqueous garlic extract (prepared from fresh garlic)	Male albino rat	- 10 days - Intragastrically 2 ml/100g body weight	*(endpoints: blood collection, histopathological examination : kidney and liver)* - ↑ urea - ↑ D-aspartate aminotransferase - ↓ alkaline phosphatase - no significant abnormalities morphologically in liver and kidney histological changes in liver : + focal area of hepatic cell necrosis, with infiltration by inflammatory cells, + moderate chronic inflammatory infiltrate in portal area and +focal kupfer cell hyperplasia
Nakagawa *et al.*, 1980 (cited in ESCOP, 2003)	Garlic juice	Female rat	- 21 days - oral route 5 ml/kg	*(endpoints : body weight, histopathological examination of stomach, liver spleen, adrenal glands)* - 5 rats died of the serious stomach injury in 21 days - body weight retardation (caused by the stomach injury) - stomach injury : congestion, hemorrhage, oedema, necrosis, ulceration, cell infiltration, hyperkeratosis, desquamation of the epithelium - swelling of the liver - hypertrophy of the spleen and the adrenal glands - ↓ erythrocytes after 3 and 8 days
Banerjee *et al.*, 2001	Fresh garlic homogenate	Wistar albino rat	- 30 days - oral route (gavage) 250, 500 and 1000 mg/kg/day	*(endpoints: biochemical parameters, histology : liver and kidney)* 250 mg/kg/day : an increase in endogenous anti-oxydant, particularly Superoxide dismutase (SOD), in liver and kidneys, along with reduction in thiobarbituric acid reactive substances (TBARS), glutathione peroxidise (GPx) 500 and 1000 mg/kg/day: significantly reduced endogenous antioxydants catalase (CAT) et SOD)

Ref.	Formulation	Species	Duration, route, dose	Noteworthy findings
				1000mg/kg/day: histopathological and ultrastructural changes in liver (large aeras of necrosis with hemorrhage and neutrophil infiltration) and kidney (marked interstitial nephritis with acute and chronic inflammation, the glomeruli shows a mild to moderate increase in mesengial cellularity with focal neutrophil infiltration
Shashikanth *et al.,* 1986 (cited in ESCOP, 2003)	Garlic extract (type of extract not specified)	Albino rat	- 4 weeks - oral route 2000 mg/kg in diet	*(endpoints: total protein content, caecal content analysis)* - growth retardation - ↓ gut flora - ↓ serum protein, - altered albumin-globulin ratio
Sumioyoshi *et al.,* 1984	Garlic extract (type of extract not specified)	Wistar rat	- 6 months - oral route 60, 200, 600 and 2000 mg/kg 5 times a week	*(endpoints : body weight, clinical pathology, histopathological examinations (organs and tissues not detailed):* No toxic signs
Non-rodent studies				
Torrescasana, 1946(mentioned in FDA evaluation for the GRAS status, 1973)	Partially purified aqueous alcohol extract of garlic bulbs	Guinea pig	- duration not known - oral route Authors reported the dose as equivalent of garlic unit per kg, equivalent of 40 g of garlic per kg	*(endpoint not detailed)* ↓ loss of weight
Sanfilippo, 1946 (mentioned in FDA evaluation for the GRAS status, 1973)	Garlic juice	Guinea pig Dog	- duration not known - oral route 1 cc/kg	*(endpoint not detailed)* ↑ [Ca] with a peak between 14 and 28 days b ut became normal after 2 months

Ref.	Formulation	Species	Duration, route, dose	Noteworthy findings
Carl, 1939 (mentioned in FDA evaluation for the GRAS status, 1973)	Fresh garlic	10 Guinea pigs	- duration not known - oral route 5 to 20% in diet	*(endpoint not detailed)* 6 animals died within 28 days

Assessor's comment

The studies mentioned in literature on garlic extracts and garlic juices targeted generally liver and kidneys and occasionally stomach. Even if, the findings suggest liver and kidneys are target organs, nevertheless, it must be noticed no study is used to evaluate the toxicity on all organs in exposed animals.

Two old studies on garlic powder were reported in report of evaluation of health aspects of garlic and oil of garlic as food ingredient which was written by FDA in February 1973. The authors (Jubb, 1947) reported that in one of these studies "it was noted on a diet of 5% dehydrated garlic, the second generation were sterile". The studies are very old and not well-documented, the duration is not known and the endpoints are not detailed.

More recent studies published by Dixit and Joshi (1982) and Hammami et al., (2008 and 2009) were designed to evaluate the effect of garlic preparation on fertility. The studies report testicular toxicity shown by decreased testosterone levels associated with an increase in LH serum levels, altered testicular function, lowered / arrest of spermatogenesis, degenerating seminiferous tubules. This toxicity occurs concomitantly with an effect of garlic on the Leydig cells and on the Sertoli cells. A NOAEL was not determined for garlic powder.

In the Dixit and Joshi (1982) reference, after oral administration of garlic powder, the LOEL is about 300 mg/kg/day. Therefore the Human Equivalent Dose (300/6.2 for rat) is 50 mg/kg/day garlic powder (about 2.5g/day for adult of 50kg). Consequently these effects on male fertility were observed at approximatively twice the maximal human daily dose. A potential impact on male fertility cannot be excluded. This finding will be reported in section 5.3.

3.3.3. Genotoxicity

The genotoxic potential was studied and reported in references from 1984 to 2007. Garlic powder, garlic extracts and its constituents (diallyl sulfide and diallyl disulfide) were evaluated for mutagenic potential in the bacteria and mammalian microsome mutagenicity test and for chromosomal aberrations induction in *in vivo* experiments.

The available data are summarised in Table 7.

Table 7: summary of genotoxicity studies

Ref.	Formulation	Type of test	Test system	Concentrations, metabolising system	Results
Garlic preparations					
Abraham *et al.*, 1984	Garlic powder in 3% gum Arabic	Chromosomal aberrations *in vivo*	Mouse, micronuclei in bone marrow	0, 2.5, 5.0, 7.5 g/kg 2 males+2 females/dose Oral administration	- Negative
Das *et al.*, 1996	Aqueous crude extract of fresh garlic	Chromosomal aberrations *in vivo*	Mouse, chromosomal aberrations and damaged cells induced in bone marrow	25, 50, 100 mg/kg Daily by gavage up to 60 days	- Higher concentrations of garlic extract are clastogenic
Yoshida *et al.*, 1984	Alcohol extract of garlic	Gene mutation in bacteria	No detailed	No detailed	- Negative
Schimmer *et al.*, 1994	Tincture of garlic bulbs	Gene mutation in bacteria	*Salmonella typhimurium* strains TA98 and TA100	Maximal dose tested : 160 µl/plate +/- S9	- Negative
Yoshida *et al.*, 1984	Fresh juice of garlic	Gene mutation in bacteria	- No detailed	- No detailed	- Negative
Yoshida *et al.*, 1984	Garlic juice	Chromosomal aberrations *in vivo*	Mouse, Chinese hamster	No detailed	Dose dependent increase of micronucleated cells and polychromatocytes on the bone marrow cells

Ref.	Formulation	Type of test	Test system	Concentrations, metabolising system	Results
Charles *et al.,* 2002	Garlic juice (peeled prior to juicing)	Chromosomal aberrations *in vitro*	Cell line CHO-K$_1$-BH$_4$	0.05% (v/v) +/- S9 Cytotoxicity Chromosomal aberration : chromosome and chromatid gaps, breaks and exchanges	- Significant inhibition of cellular growth at concentrations as low as 0.05% (v/v) - In absence of S9, significant levels of chromosomal damage relative to control treatments, predominantly in the form of chromatid breaks and exchanges (garlic 0.05%)
Kalantari *et al.,* 2007	Garlic drop	Chromosomal aberrations *in vivo*	Mouse, micronuclei in peripherical blood	2.5, 5 and 10 mg/kg 5 wistar albino male mice 2 administrations at 24h intervals (route not specified)	- Equivocal test response : Garlic drops showed a significant dose-dependent genotoxic effect compared to the negative control group but "not significant" compared to the historical negative control group
Garlic constituents					
Musk *et al.,* 1997	Diallyl sulfide (DAS) Diallyl disulfide (DDS)	Chromosomal aberrations *in vitro*	Chinese hamster ovary cell line	DDS : 2 to 25 µg/ml DAS : 200 to 600 µg/ml	- Induce both chromosome aberrations and sister chromatid exchanges : DDS : activity at concentration below 10 µg/ml DAS : activity at concentration 300 µg/ml and above

Assessor's comment

Even if the only one study on garlic powder (Abraham, 1984) reports negative results, the exposure of animals was not checked; so this result must be taken into account carefully. However, some garlic preparations or its constituents induce chromosome aberrations.

No conventional genotoxicity studies have been reported.

3.3.4. Carcinogenicity

No conventional carcinogenicity study is available.

Assessor's comment

No conventional carcinogenicity studies are available. The human carcinogenic risk is not known considering the endocrine disruptor potential.

3.3.5. Reproductive and developmental toxicity

No conventional reprotoxicity study is available.

An *in vitro* evaluation of teratogenic potential is reported by Charles *et al.,* (2002) on limb bud micromass of 11 days old mouse embryos. Cytotoxicity and differentiation into chrondrocytes was assessed. Garlic juice is considered non specific inhibitors with ratios (IC50Differentiation/IC50cytotoxicity) of approximately 1, meaning that cytotoxicity and inhibition of differentiation appeared to parallel each other.

Assessor's comment

No conventional reprotoxicity study is available; however in repeated toxicity studies, spermatogenesis impairment was reported (see above). According to the guideline ICH S5 (R2) for the detection to reproduction for medicinal products and toxicity to male fertility, "compounds inducing selective effects on male reproduction are rare; mating with females is an insensitive means of detecting effects on spermatogenesis; good pathological and histopathological examination of the male reproductive organs provides a more sensitive and quicker means of detecting effects on spermatogenesis.

3.3.6. Local tolerance

No study available

3.3.7. Other special studies

One study focused on the impact of garlic juice on estrogen receptor in MCF-7 cells was reported in literature (Charles, 2002). The response to the garlic juice was not consistently elevated enough above background (10% water) to be convincingly classified as estrogenic.

3.4. Overall conclusions on non-clinical data

Pharmacology

Numerous *in vitro* and *in vivo* (rats, mice, rabbits and dogs) studies were published with garlic powder, different extracts and with some isolated constituents of garlic. Experiments on lipids, on blood pressure, on platelet aggregation and anti-oxidant activity may contribute to the long-standing use of garlic in the indication of cardiovascular prevention.

Some controversial results are reported in non clinical studies, one of possible explanations for the discrepancy may stem from the different constituents of garlic or garlic preparations used and different durations and design study.

The antilipidemic, antihypertensive, antithrombotic and antiglycaemic effects observed in animal studies have been largely studied in human studies.

No safety pharmacology data were available.

Pharmacokinetics

No pharmacokinetics data about garlic powder and garlic preparations were available.

An *in vitro* study reported that garlic can affect cytochrome P-450 2C9, 2C19, 2D6 and 3A4, 3A5, 3A7 mediated-metabolism.

Toxicity

The available toxicological data are rather limited. The acute toxicity of garlic powder remained unknown. The acute of toxicity in published data seems to be subject of controversy; in one study, garlic oil has been shown to be lethal at 100 mg/kg while in other study the LD50 of aqueous extract is reported at over 3000 mg/kg.

Two old studies on garlic powder were reported in report of evaluation of health aspects of garlic and oil of garlic as food ingredient which was written by FDA in February 1973. The authors (Jubb, 1947) reported that in one of these studies "it was noted on a diet of 5% dehydrated garlic, the second generation were sterile". The studies are very old and not well-documented, the duration is not known and the endpoints are not detailed.

More recent studies published by Dixit and Joshi (1982) and Hammami *et al.,* (2008 and 2009) were designed to evaluate the effect of garlic preparation on fertility. The studies report testicular toxicity shown by decreased testosterone levels associated with an increase in LH serum levels, altered testicular function, lowered / arrest of spermatogenesis, degenerating seminiferous tubules. This toxicity occurs concomitantly with an effect of garlic on the Leydig cells and on the Sertoli cells. A NOAEL was not determined for garlic powder.

In the Dixit and Joshi (1982) reference, after oral administration of garlic powder, the LOEL is about 300 mg/kg/day. Therefore the Human Equivalent Dose (300/6.2 for rat) is 50 mg/kg/day garlic powder (about 2.5g/day for adult of 50kg). Consequently these effects on male fertility were observed at approximatively twice the maximal human daily dose. A potential impact on male fertility cannot be excluded. This finding will be reported in section 5.3.

An *in vivo* micronucleus assay on garlic powder after oral administration reports negative results but the exposure of animals was not checked; thus this result must be taken into account carefully. However, some garlic preparations or its constituents induce chromosome aberrations.

No conventional genotoxicity, carcinogenicity and reproduction toxicity studies are available.

4. Clinical Data

4.1. Clinical pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

There are dozens of brands of garlic products on the market that provide a convenient way to obtain the health benefits of garlic. They can be classified into four groups: garlic essential oil, garlic oil macerate, garlic powder, and garlic extract. The chemistry of garlic is quite complicated and different types of processing produce products that are more than just preparations in different forms.

The various forms also differ in their ingredients, effects, and toxicities.

4.1.1.1. *Pharmacodynamic of relevant herbal preparations*

The garlic variety and manufacturing process are important considerations when choosing a garlic supplement, since products with different biologically active compounds, effects and toxicities can be originated.

The main type of products and characteristics of garlic products on the market are the following (Amagase, 2006):

Table 8: Main type of products and characteristics of garlic products on the market

Type of products	Main compounds and characteristics
Garlic Essential Oil	Only 1% of Oil-soluble sulfur compounds (DAS, DADS, etc.) in 99% vegetable oil No water-soluble fraction No allicin* Not well-standardised No safety data
Garlic oil macerate Oil	Soluble sulfur compounds and alliin No allicin* Not well-standardised No safety data
Garlic powder	Alliin and a small amount of oil-soluble sulfur compounds No allicin* Not well-standardised Results on cholesterol is not consistent. No safety data
Aged garlic extract (AGE)	Mainly water-soluble compounds (S-allylcysteine, SAMC, saponins, etc.) Standardised with S-allylcysteine Small amount of oil-soluble sulphur compounds Various beneficial effects Well-established safety Heavily researched (4001 papers)

* Allicin is a highly unstable and reactive compound that rapidly decomposes to other compounds. For this reason no garlic product on the market contains a detectable amount of allicin (0.1 mg/g).

Sulphur-containing compounds in commercial garlic preparations vary, depending on their manufacturing processes:

4.1.1.2. *Pharmacodynamic data*

Antiatherogenic and lipid-lowering effects

According to ESCOP monograph, inhibition of cholesterol biosynthesis by allicin and ajoene was evaluated in the human liver cell line HepG2. Both allicin and ajoene inhibited sterol biosynthesis with IC50 values of 7 and 9 µM respectively. The inhibition was exerted at the level of HMG-CoA-reductase (Gebhardt *et al.*, 1994).

In addition, during a 24-hour incubation period, garlic powder significantly reduced the level of cholesteryl esters by 26% and free cholesterol by 32% in cells of atherosclerotic plaques of human aorta ($p < 0.05$) and inhibited their proliferative activity by 55% at 1 mg/ml (Orekhov et al., 1995).

Antihypertensive effects

Blood pressure reducing properties of garlic have been linked to its hydrogen sulphide production (Benavides et al., 2007) and allicin content – liberated from alliin and the enzyme alliinase – which has angiotensin II inhibiting and vasodilating effects, as shown in animal and human cell studies.

Antiglycaemic effects

No data available

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Little data is available from clinical studies concerning the absorption, metabolism, and distribution of garlic-derived compounds.

Organo-sulfur compounds

The pharmacokinetic destiny of organo-sulfur compounds seems to be very diverse and includes numerous spontaneous transformation reactions, which take place during garlic processing, shelf-life or in the body after consumption. The final metabolites are exhalted (for lipophilic and volatile garlic compounds, final metabolites include allyl methyl sulfone, acetone) or excreted in the urine (for water soluble garlic compounds).

Two classes of organosulfur compounds are found in whole garlic cloves: (1) gamma glutamylcysteines, and (2) cysteine sulfoxides. Allylcysteine sulfoxide (alliin) accounts for approximately 80% of the cysteine sulfoxides in garlic.

When raw garlic cloves are crushed, chopped, or chewed, an enzyme known as alliinase is released. Alliinase catalyzes the formation of sulfenic acids from cysteine sulfoxides. Sulfenic acids spontaneously react with each other to form unstable compounds called thiosulfinates. In the case of alliin, the resulting sulfenic acids react with each other to form a thiosulfinate known as allicin.

The formation of thiosulfinates is very rapid and has been found to be complete within 10-60 seconds of crushing garlic. Allicin breaks down *in vitro* to form a variety of fat-soluble organosulfur compounds, including diallyl trisulfide (DATS), diallyl disulfide (DADS), and diallyl sulfide (DAS), or in the presence of oil or organic solvents, ajoene and vinyldithiins:
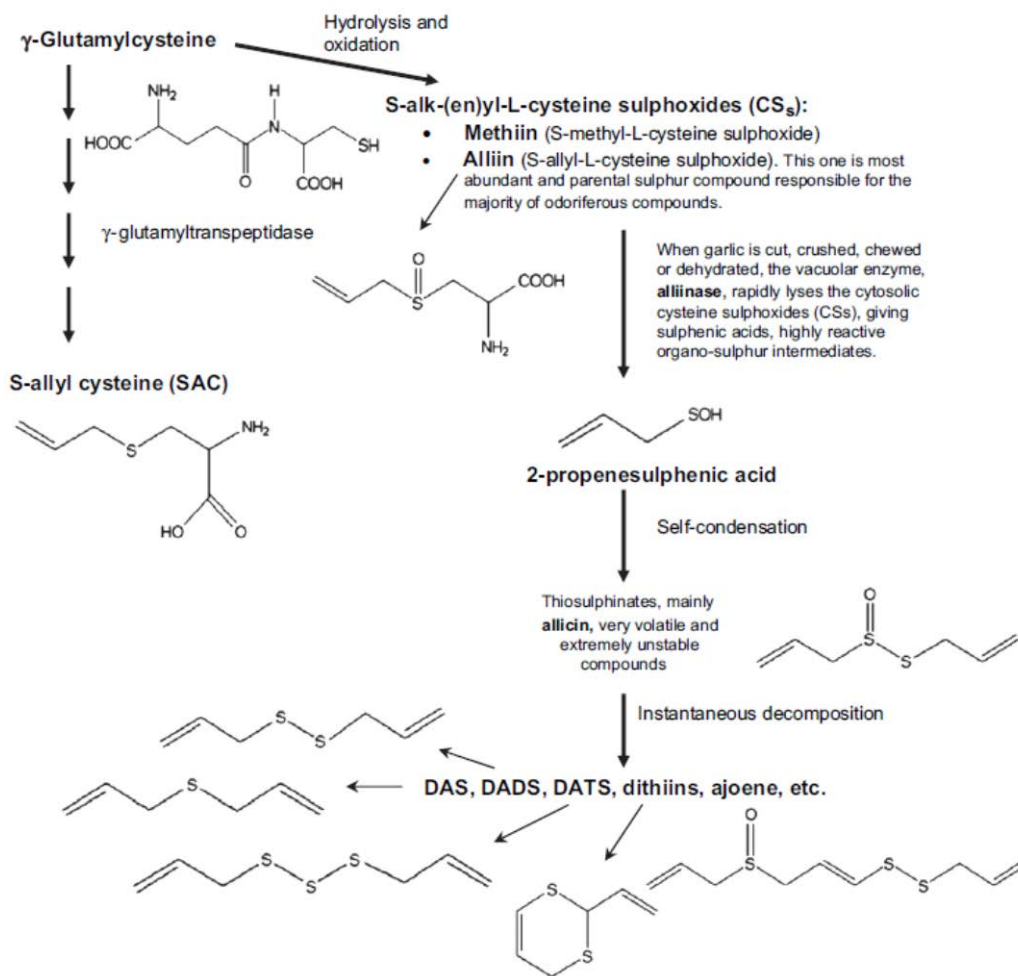
Figure 2: Formation of organo-sulphur compounds during metabolic pathways in processed garlic (Corzo-Martinez, 2007)

Allicin

Several studies have revealed that the bioavaibility of allicin is poor due to its great instability, not being detected in the blood or urine after ingestion of raw garlic. Currently, it is well known that allicin is simply a transiet compound that is rapidly decomposed to other products.

Flavonoids compounds

Flavonoids are poorly absorbed after oral intake and are extensively metabolised in pre-systemic to glucuronides and sulfates, which are excreted in gastrointestinal tract or bile (Prasain JK 2007)

Sapogenins and saponins

No available data

Selenium compounds and fructosamines

No available data

4.2. *Clinical efficacy*

4.2.1. Dose response studies

Dehydrated garlic powder

The most commonly used doses ranged from of 600-900 mg/day and provided 3,600-5,400 mcg/day of potential allicin, but a dose-response relation has not yet been clearly demonstrated.

Aged garlic extract

AGE has a wide range of effectiveness based upon clinical studies. AGE with a dosage range of 1–7.2 g/d has been used to lower plasma cholesterol in humans. Studies show that as little as 1.8 g to as much as 10 g/d of AGE is effective in enhancing human immune responses (Amagase, 2006).

Interestingly, no severe toxic side effects were reported in these clinical studies even at high dosages.

4.2.2. Clinical studies (case studies and clinical trials)

The efficacy of *Allium sativum* has been proposed in the different following indications. A review of the literature for each indication has been performed.

a. *Antilipidemic effects*

The literature of clinical trials exploring the antilipidemic effects of garlic is abundant.

Standardised and non standardised preparations (mixed data)

The WHO monograph : according to the WHO monograph, a meta-analysis of the effects of Bulbus Allii Sativi on serum lipids and lipoproteins reviewed 25 randomised, controlled trials (published and unpublished) (Silagy and al., 1994) and selected 16 with data from 952 subjects to include in the analysis. Fourteen of the trials used a parallel group design, and the remaining two were cross-over studies. Two of the studies were conducted in an open-label fashion, two others were single-blind, and the remainder were double-blind. The total daily dose of garlic was 600–900mg of dried garlic powder, or 10g of raw garlic, or 18 mg of garlic oil, or aged garlic extracts (dosage not stated). The median duration of the therapy was 12 weeks. Overall, the subjects receiving garlic supplementation (powder or non-powder) showed a 12% reduction (average) in total cholesterol and a 13% reduction (powder only) in serum triglycerides. Meta-analysis of the clinical studies confirmed the lipid-lowering action of garlic. However, the authors concluded that the overall quality of the clinical trials was poor and that favourable results of better designed clinical studies should be available before garlic can be routinely recommended as a lipid-lowering agent. However, current available data support the hypothesis that garlic therapy is at least beneficial. Another meta-analysis of the controlled trials of garlic effects on total serum cholesterol reached similar conclusions (Warshafsky and al., 1993). A systematic review of the lipid-lowering potential of a dried garlic powder preparation in eight studies with 500 subjects had similar findings (Brosche, 1990). In seven of the eight studies reviewed, a daily dose of 600–900mg of garlic powder reduced serum cholesterol and triglyceride levels by 5–20%. The review concluded that garlic powder preparations do have lipid-lowering potential.

ESCOP monograph: According to ESCOP monograph, the effect of garlic on total serum cholesterol has been assessed in four meta-analyses (Neil and al., 1994; Silagy and al., 1994; Stevinson and al., 2000; Warshafsky and al., 1993). In all of them, it was concluded that the available data showed garlic to be superior to placebo in reducing total cholesterol with garlic powder tablets at doses of 0.6 to 1.2 g; however, only a modest effect was indicated from one meta-analysis (Stevinson and al, 2000).

According to the WHO monograph, the overall quality of the clinical trials performed before 2000 is poor, notably due to the low number of patients included. Only two studies have randomized more than one hundred patient: Mader (1990) and Neil (1996). A brief description of these studies is provided here.

Gardner (2007): This trial compares the effect of raw garlic and 2 commonly used garlic supplements versus placebo on cholesterol concentrations in adults with moderate hypercholesterolemia. In this parallel-design trial, 192 adults with low-density lipoprotein cholesterol (LDL-C) concentrations of 130 to 190 mg/dL (3.36-4.91 mmol/L) were randomly assigned to 1 of the following 4 treatment arms: raw garlic, powdered garlic supplement, aged garlic extract supplement, or placebo.

Garlic product doses equivalent to an average-sized garlic clove were consumed 6 d/wk for 6 months as follow: 4.0 g of blended raw garlic (an average-sized clove crushed in a blender; hereafter, raw garlic), 4 Garlicin tablets (=powdered garlic supplement) at twice the recommended dose, 6 Kyolic tablets (aged garlic extract) at 1,5-3 times (i.e. 1.8 g/day) the recommended dose , or 4 or 6 placebo tablets. The primary study outcome was LDL-C concentration. Fasting plasma lipid concentrations were assessed monthly.

There were no statistically significant effects of the 3 forms of garlic on LDL-C concentrations. The 6-month mean (SD) changes in LDL-C concentrations were +0.4 (19.3) mg/dL (+0.01 [0.50] mmol/L), +3.2 (17.2) mg/dL (+0.08 [0.44] mmol/L), +0.2 (17.8) mg/ dL(+0.005 [0.46] mmol/L),and−3.9 (16.5)mg/dL(−0.10 [0.43]mmol/L) for raw garlic, powdered supplement, aged extract supplement, and placebo, respectively. There were no statistically significant effects on high-density lipoprotein cholesterol, triglyceride levels, or total cholesterol– high-density lipoprotein cholesterol ratio.

This study is unusual because of the effort made by the investigators to document the bioavailability of the allyl thiosulfinates by measuring breath allyl methyl sulphide before the trial. The authors documented the phytochemical stability over time of each preparation, showing the concentrations of 14 sulfur and 2 non sulphur at 3, 6, 12, 18 and 24 months. They also showed that the allyl thiosulfinates were nearly the same for raw garlic and powdered garlic, although there were quantitative and qualitative differences for the aged garlic (Charlson and al., 2007).

This study is a well-designed trial in terms of blinding, compliance, randomization, and power calculations, testing three different preparation of garlic with posology superior to recommended doses. No significant effects on LDL-C or other plasma lipid concentrations in adults with moderate hypercholesterolemia were observed.

Meta-analysis

Many systemic meta-analytic reviews have investigated the effectiveness and properties of garlic on cholesterol and triglycerides (Banerjee and al, 2002; Alder and al., 2003; Reinhart and al; 2009; Khoo and al., 2009; Tsai and al., 2012, Zeng et al., 2012, Ried et al., 2013).

The results of the meta-analysis on lipid are conflicting:

Table 9: Results of meta-analyses on the effect of garlic on lipids parameters (Ried, 2013)

Reference	No. of trials in meta-analysis	Mean difference (SE) between garlic and control group (mg/dL)			
		Total cholesterol	LDL cholesterol	HDL cholesterol	Triglycerides
Warshafsky et al. (1993)[13]	6	−23.0 (6.0)	NR	NR	
Silagy & Neil (1994)[14]	14	−29.8 (4.6)	NR	−1.6 (0.4)	27.5 (15.9)
Stevinson et al. (2000)[15]	13	−15.7 (10.0)	−6.6 (6.2)	2.7 (1.2)	NR
Ackermann et al. (2001)[16]	33[a]	−7.2 (6.0)	6.2 (5.4)	0.9 (1.8)	−19.1 (10.6)
Khoo & Aziz (2009)[17]	12	−1.6 (4.3)	0.4 (3.9)	0.4 (1.6)	−4.4 (10.6)
Reinhart et al. (2009)[18]	29	−7.4 (5.4)	2.3 (3.6)	1.0 (0.9)	−9.8 (7.1)
Zeng et al. (2012)[19]	26	−10.8 (6.6)	0 (2.7)	0.4 (0.4)	−11.5 (6.2)
Ried et al. (2013)[present review]	39	−15.2 (5.5)	−6.4 (5.4)	1.8 (1.2)	−5.5 (8.7)

Assessor's comments

The old meta-analysis published before 2000 have suggested an effect on lipid parameter whereas the more recent reviews, published between 2001 and 2012, have suggested a more modest role for garlic on plasma lipid levels or no effects.

The Rapporteur's will particularly describe the meta-analysis from Khoo and al., 2009 as it seems to be the most restrictive meta-analysis and the meta-analysis from Ried et al., 2013 as it is the most extensive analysis in terms of number of clinical trials included.

Khoo et al., 2009: A comprehensive search of the Cochrane Library, MEDLINE, EMBASE, electronic publishing sites, reference lists of relevant papers and manual searches of relevant journals from inception to March 2008 was carried out. To evaluate the effects of garlic on cholesterol levels in both healthy and hypercholesterolaemic subjects, randomised controlled trials of garlic ranging from 11 to 24 weeks in duration were included. Thirteen trials including 1056 subjects were eligible for the meta-analysis.

Garlic therapy did not produce any statistically significant reduction in serum total cholesterol level (mean difference, -0.04 mmol/L; 95% CI -0.15 to 0.07 mmol/L), LDL-cholesterol level (mean difference, 0.01 mmol/L; 95% CI -0.10 to 0.11 mmol/L), triglycerides level (mean difference, -0.05 mmol/L; 95% CI -0.17 to 0.06 mmol/L) or apolipoprotein B level (mean difference, -0.02 g/L; 95% CI -0.03 to 0.001 g/L). There was no difference between garlic and placebo on HDL-cholesterol level (mean difference, 0.01 mmol/L; 95% CI -0.03 to 0.05 mmol/L). Results appear similar in healthy patients and hypercholesterolaemic patients.

Assessor's comments

The review question was clearly stated and well supported by study inclusion criteria. The literature search was comprehensive and efforts were made to find unpublished studies. There were stated to be no language restrictions. Validity assessment was performed using a validated scale and results were presented appropriately. Study details were well reported and pooling seemed appropriate. Heterogeneity was assessed, although reasons for heterogeneity were not explored.

Given the substantial clinical heterogeneity of the populations enrolled (hypercholesterolaemic patients and healthy subjects) and the differences in garlic dose and type across the included studies, the reviewers' decision to pool the results in a meta-analysis may not have been appropriate.

The authors conclude that the available evidence from randomized controlled trial does not demonstrate any beneficial effects on serum cholesterol. A separate analyse was conducted for comparisons of garlic preparation in healthy and hypercholesterolaemic subjects showing similar results.

Ried (2013): TheMedline and Cochrane databases and Google Scholar were searched for randomised, placebo-controlled human trials investigating the effect of garlic on cholesterol and published between 1955 and December 2011 in English or German using the following search terms: garlic, allium sativum, allicin, cholesterol, hyperlipidemia, and lipid. Trials were included in the meta-analysis if they were ≥2 weeks in duration, contained a true placebo control group, were conducted in adult subjects, and tested garlic as a single active substance. Data on total serum cholesterol LDL cholesterol, HDL cholesterol, and triglyceride levels at baseline and at the end of the trial were collated.

Meta-analyses of all eligible trials on the effect of garlic on total cholesterol (TC), LDL cholesterol, HDL cholesterol, and triglyceride were conducted, as were subgroup analyses by duration (2–8 weeks [short] or >8 weeks [long]), by TC baseline (<200 mg/dL or >200 mg/ dL), by type of garlic supplement (garlic powder, entericcoated garlic powder, aged garlic extract, garlic oil, or raw garlic), and by industry funding.

A total of 39 trials fit inclusion criteria for meta-analysis, of which 37 reported sufficient data on total cholesterol levels, 26 reported LDL cholesterol data, 30 reported HDL cholesterol data, and 32 reported triglyceride data.

Assessor's comments
The review question was clear with regard to the eligible study designs, interventions and outcome measures. Relevant databases were searched for studies published in English or German, so there was a risk of language bias.

The characteristics of trials included in the meta-analysis are shown in table 6

Table 10: Characteristics of trials included in the meta-analysis performed by Ried (2013)

Reference	No. of subjects (garlic/ control)	Study design	Garlic type	Brand	Dose of product (mg/day)	No. of tablets or capsules[a] per day	Dose of active ingredient per day	Duration of trial (weeks)	Total cholesterol, garlic group Baseline (mg/dL)	End (mg/dL)	Total cholesterol, control group Baseline (mg/dL)	End (mg/dL)
Bhushan et al. (1979)[24]	15/10	p	RG	NCP	10,000	NA	NR	8.5	223	190	206	205
Bordia (1981)[25]	33/29	p	GO	NCP	0.25/kg	2	NR	40	300	230	280	280
Barrie et al. (1987)[26]	20/20	c	GO	NCP	18	NR	NR	4	195	180	193	190
Lau et al. (1987)[27]	15/12	p	AGE	Kyolic[e]	1,000	4	1.2 mg SAC	24	313	262	303	292
Sitprija et al. (1987)[28]	17/16	p	GP	NCP	700	2	NR	4	219	229	237	240
Plengvidhya et al. (1988)[29]	16/14	p	GP	NCP	700	2	NR	8	266	234	295	269
Auer et al. (1990)[30]	24/23	p	GP	Kwai[e]	600	2	7.8 mg alliin[b]	12	268	230	267	247
Mader (1990)[31]	111/110	p	GP	Kwai	800	4	10.4 mg alliin	16	266	235	262	255
Vorberg & Schneider (1990)[32]	20/20	p	GP	Kwai[e]	900	NR	11.7 mg alliin[b]	16	294	233	288	278
Gadkari & Joshi (1991)[33]	30/20	p	RG	NCP	10,000	NA	NR	8.5	213	180	212	212
Rotzsch et al. (1992)[34]	12/12	p	GP	Kwai[e]	900	3	11.7 mg alliin[b]	6	NR	NR	NR	NR
Jain et al. (1993)[35]	20/22	p	GP	Kwai[e]	900	3	11.7 mg alliin	12	262	247	276	274
Kiesewetter et al. (1993)[36]	32/32	p	GP	Kwai[e]	800	4	10.4 mg alliin[b]	12	267	234	264	253
Phelps & Harris (1993)[37]	10/10	c	GP	Kwai[e]	600	6	7.8 mg alliin[b]	2	176	175	174	173
DeA Santos & Gruenwald (1993)[38]	25/27	p	GP	Kwai[e]	900	NR	11.7 mg alliin	24	268	244	273	261
Saradeth et al. (1994)[39]	31/37	p	GP	Kwai[e]	600	NR	7.8 mg alliin	15	223	214	217	218
Simons et al. (1995)[40]	28/28	c	GP	Kwai[e]	900	3	11.7 mg alliin[b]	12	260	253	260	251
Neil et al. (1996)[41]	57/58	p	GP	Kwai[e]	900	3	11.7 mg alliin	24	269	267	270	273
Adler & Holub (1997)[42]	12/11	p	GP	Kwai[e]	900	3	11.7 mg alliin[b]	12	253	224	250	251
Yeh et al. (1997)[43]	16/16	p	AGE	Kyolic[e]	7,200	9	NR	20	246	228	243	245
Berthold et al. (1998)[44]	25/25	c	GO	Tegra[e]	10	NR	4,000 U allicin eq	12	291	NR	291	NR
Bordia et al. (1998)[45]	30/30	p	GO	NCP	4[c]	4	NR	12	253	201	253	249
Isaacsohn et al. (1998)[46]	28/22	p	GP	Kwai[e]	900	3	11.7 mg alliin[b]	12	274	279	250	250
Rahmani et al. (1999), trial arm A[47]	30/22	p	GP	Garlet[e]	1,200	3	NR	12	274	268	267	267
Rahmani et al. (1999), trial arm B[47]	21/22	p	GP	Garlet[e]	2,400	6	NR	12	259	238	267	267
Rahmani et al. (1999) trial arm C[47]	20/22	p	GP	Garlet[e]	3,600	9	NR	12	258	255	267	267
Superko & Krauss (2000)[48]	25/25	p	GP	Kwai[e]	900	3	11.7 mg alliin[b]	12	250	278	239	261
Zhang et al. (2000)[49]	14/13	p	GO	Cardiomax[e]	12.3	3	NR	16	186	184	178	175
Kannar et al. (2001)[50]	20/23	p	GP	NCP	880	4	9.6 mg allicin pt	12	286	245	275	280
Zhang et al. (2001), trial arm A[51]	19/21	p	GO	Cardiomax[e]	9	2	8.2 mg allyl sulfide	11	186	176	190	188
Zhang et al. (2001), trial arm B[51]	20/21	p	GP	Garlicin[e]	1,000	2	7.8 mg allicin pt	11	166	163	190	188
Peleg et al. (2003)[52]	13/20	p	GP	Inod'All	22,400[d]	4	22.4 mg alliin	16	263	260	275	268
Satitvipawee et al. (2003)[53]	70/76	p	GP	NCP	333	1	5.6 allicin pt	12	257	255	265	263
Budoff et al. (2004)[54]	9/10	p	AGE	Kyolic[e]	1,220	4 ml[e]	1.2 mg SAC	48	176	183	199	212
Tanamai et al. (2004)[55]	45/45	p	GP	NCP	900	NR	5 mg allicin pt	13	284	266	284	267
Ashraf et al. (2005)[56]	35/35	p	GP	Garlex[e]	600	2	15.6 mg alliin	12	228	201	220	218
Williams et al. (2005)[57]	15/15	c	AGE	Kyolic[e]	2,400	4	2 mg SAC[b]	2	166	166	170	166
Macan et al. (2006)[58]	22/26	p	AGE	Kyolic[e]	3,050	10 ml[e]	14.7 mg SAC	12	184	192	184	180
Gardner et al. (2007), trial arm A[59]	42/43	p	RG	NCP	4,000	NA	NR	24	NR	NR	NR	NR
Gardner et al. (2007), trial arm B[59]	41/43	p	GP	Garlicin[e]	4,000	4	3.2 mg allicin pt	24	NR	NR	NR	NR
Gardner et al. (2007), trial arm C[59]	42/43	p	AGE	Kyolic[e]	4,000	6	1.5 mg SAC	24	NR	NR	NR	NR
Sobenin et al. (2008)[60]	23/19	p	GP	Allicor[e]	600	2	7.8 mg allicin pt	12	270	248	273	280
Sobenin et al. (2010)[61]	26/25	p	GP	Allicor[e]	300	2	3.9 mg allicin pt	52	270	236	253	242
Han et al. (2011)[20]	22/22	p	GP	NCP	1,000	2	0.75 mg SAC	8	201	199	183	176

[a] Garlic powder, aged garlic extract powder, or garlic oil.
[b] Estimation based on standardized formula; active ingredient not reported in publication.
[c] Estimate of volume of garlic oil extracted from 2 g of raw garlic.
[d] Dosage, as reported in Peleg et al.,[52] likely too high by a factor of 10.
[e] Volume of aged garlic extract liquid.

Abbreviations: AGE, aged garlic extract; c, crossover trials; eq, equivalent (allicin equivalent measured by adding water to garlic product containing allicin and alliinase); GO, garlic oil; GP, garlic powder; NA, not applicable; NCP, noncommercial product; NR, not reported; p, parallel trials; pt, potential; RG, raw garlic; SAC, S-allylcysteine.

Assessor's comments

The majority of trials use garlic powder (27 of 37 trials included). Aged garlic extract has been used in only 6 trials with a low number of patients included.

Only two trials (Zhang (2001) and Gardner (2007)) have compared different types of garlic in a clinical trial. Regarding Zhang (2001), the comparison is indirect as it is a randomized placebo-controlled trial of 11 weeks including 51 healthy, normo-lipidaemic volunteers assigned to garlic oil capsules or placebo with an additional non-randomized arm of 27 patients assigned to garlic powder. The difference on cholesterol was not significant between oil garlic, garlic powder and placebo.

There is no comparative study with accepted pharmacotherapy for the treatment of hypercholesterolemia.

Results on total cholesterol: Meta-analysis of 37 trials revealed a significant cholesterol-lowering effect of garlic preparations compared with placebo (mean difference TC = -15.25 [95%CI: -20.72, -9.78] mg/dL; I2 = 77%; P < 0.0001, figure 3). The cholesterol-lowering treatment effect was more pronounced in trials of longer duration (subgroup>8 wks: mean difference TC = -17.20 [95%CI: -23.10, -11.30] mg/dL; I2 = 79%; P < 0.0001; n = 31), in trials of subjects with higher mean baseline TC levels (subgroupTC baseline >200 mg/dl: mean difference TC = -17.32 [95%CI: -23.48, -11.16]; I2 = 81%; P < 0.0001; n = 29).

Study	Total serum cholesterol (mg/dL)	Mean Difference [95% CI]
Bhushan et al. (1979)[24]		-31.80 [-53.58, -10.02]
Bordia (1981)[25]		-70.00 [-111.59, -28.41]
Barrie et al. (1987)[26]		-12.90 [-34.48, 8.68]
Lau et al. (1987)[27]		-34.00 [-84.37, 16.37]
Sitprija et al. (1987)[28]		7.10 [-34.55, 48.75]
Plengvidhya et al. (1988)[29]		-6.00 [-40.91, 28.91]
Auer et al. (1990)[30]		-18.00 [-37.72, 1.72]
Mader (1990)[31]		-24.20 [-34.94, -13.46]
Vorberg & Schneider (1990)[32]		-51.00 [-64.88, -37.12]
Gadkari & Joshi (1991)[33]		-33.30 [-42.55, -24.05]
Jain et al. (1993)[35]		-13.00 [-34.30, 8.30]
Kiesewetter et al. (1993)[36]		-21.90 [-44.28, 0.48]
Phelps & Harris (1993)[37]		0.00 [-29.22, 29.22]
DeASantos & Gruenwald (1993)[38]		-11.60 [-25.46, 2.26]
Saradeth et al. (1994)[39]		-9.70 [-28.42, 9.02]
Simons et al. (1995)[40]		1.90 [-8.70, 12.50]
Neil et al. (1996)[41]		-4.50 [-14.40, 5.40]
Adler & Holub (1997)[42]		-30.20 [-59.48, -0.92]
Yeh et al. (1997)[43]		-20.00 [-33.86, -6.14]
Berthold et al. (1998)[44]		3.30 [-7.48, 14.08]
Bordia et al. (1998)[45]		-47.80 [-67.48, -28.12]
Isaacsohn et al. (1998)[46]		4.50 [-10.61, 19.61]
Rahmani et al. (1999)[47] trial-arm B		-21.20 [-40.76, -1.64]
Superko & Krauss (2000)[48]		6.00 [-8.50, 20.50]
Zhang et al. (2000)[49]		0.80 [-22.52, 24.12]
Kannar et al. (2001)[50]		-46.30 [-69.76, -22.84]
Zhang et al. (2001)[51] trial-arm A		-7.80 [-27.28, 11.68]
Peleg et al. (2003)[52]		4.70 [-19.98, 29.38]
Satitvipawee et al. (2003)[53]		-0.50 [-12.00, 11.00]
Budoff et al. (2004)[54]		-5.80 [-118.42, 106.82]
Tanamai et al. (2004)[55]		-0.10 [-11.78, 11.58]
Ashraf et al. (2005)[56]		-25.25 [-36.46, -14.04]
Williams et al. (2005)[57]		3.90 [-20.09, 27.89]
Macan et al. (2006)[58]		-24.20 [-47.41, -0.99]
Sobenin et al. (2008)[60]		-29.30 [-50.84, -7.76]
Sobenin et al. (2010)[61]		-23.00 [-27.21, -18.79]
Han et al. (2011)[20]		4.10 [-18.15, 26.35]
Total (n=37 trials) I2= 77% p < 0.00001		**-15.25 [-20.72, -9.78]**

Figure 3: Meta-analysis of Ried (2013) testing the effect of garlic on total serum cholesterol

Assessor's comments

Once again, the old trials seem to show a benefit on total cholesterol whereas more recent trials show more controversial results. Whether the reduction of -15.25 mg/dL translates into any medical benefit remains unclear.

The majority of trials used garlic powder preparations (n = 24), although some used garlic oil (n = 6), aged garlic extract (n = 5), or raw garlic (n = 2). Subgroup analysis by single type of garlic preparation suggested a greater cholesterol-lowering effect for aged garlic extract than for garlic powder, and a borderline effect for garlic oil, while subgroup analysis with two studies using raw garlic is less meaningful.

Results on LDL cholesterol: Meta-analysis including all trials showed a moderate significant reduction of LDL cholesterol by garlic compared with placebo (mean difference LDL = - 6.41 [95%CI: - 11.77, -1.05] mg/dL, I2 = 75%; P = 0.02; n = 26, figure 4). Subgroup analysis by effect of garlic type on LDL cholesterol was significant for garlic powder but not for aged garlic extract.

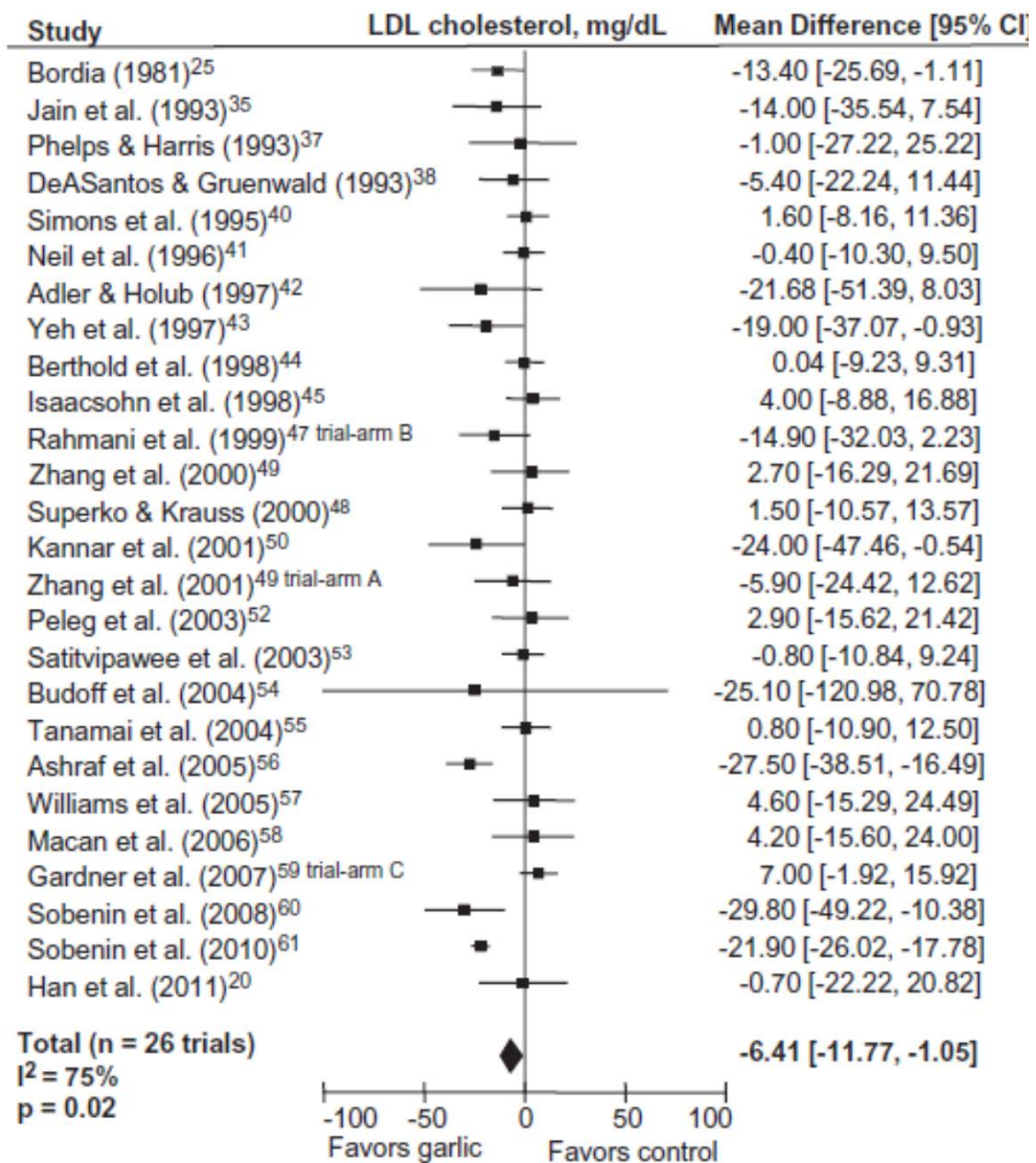| Study	LDL cholesterol, mg/dL	Mean Difference [95% CI]
Bordia (1981)[25]		-13.40 [-25.69, -1.11]
Jain et al. (1993)[35]		-14.00 [-35.54, 7.54]
Phelps & Harris (1993)[37]		-1.00 [-27.22, 25.22]
DeASantos & Gruenwald (1993)[38]		-5.40 [-22.24, 11.44]
Simons et al. (1995)[40]		1.60 [-8.16, 11.36]
Neil et al. (1996)[41]		-0.40 [-10.30, 9.50]
Adler & Holub (1997)[42]		-21.68 [-51.39, 8.03]
Yeh et al. (1997)[43]		-19.00 [-37.07, -0.93]
Berthold et al. (1998)[44]		0.04 [-9.23, 9.31]
Isaacsohn et al. (1998)[45]		4.00 [-8.88, 16.88]
Rahmani et al. (1999)[47] trial-arm B		-14.90 [-32.03, 2.23]
Zhang et al. (2000)[49]		2.70 [-16.29, 21.69]
Superko & Krauss (2000)[48]		1.50 [-10.57, 13.57]
Kannar et al. (2001)[50]		-24.00 [-47.46, -0.54]
Zhang et al. (2001)[49] trial-arm A		-5.90 [-24.42, 12.62]
Peleg et al. (2003)[52]		2.90 [-15.62, 21.42]
Satitvipawee et al. (2003)[53]		-0.80 [-10.84, 9.24]
Budoff et al. (2004)[54]		-25.10 [-120.98, 70.78]
Tanamai et al. (2004)[55]		0.80 [-10.90, 12.50]
Ashraf et al. (2005)[56]		-27.50 [-38.51, -16.49]
Williams et al. (2005)[57]		4.60 [-15.29, 24.49]
Macan et al. (2006)[58]		4.20 [-15.60, 24.00]
Gardner et al. (2007)[59] trial-arm C		7.00 [-1.92, 15.92]
Sobenin et al. (2008)[60]		-29.80 [-49.22, -10.38]
Sobenin et al. (2010)[61]		-21.90 [-26.02, -17.78]
Han et al. (2011)[20]		-0.70 [-22.22, 20.82]
Total (n = 26 trials) I2 = 75% p = 0.02		-6.41 [-11.77, -1.05]

-100 -50 0 50 100
Favors garlic   Favors control

Figure 4: Meta-analysis of Ried (2013) testing the effects of garlic on LDL cholesterol

Assessor's comments

In patients with primary hypercholesterolemia, reduction in LDL-cholesterol could be considered as the primary endpoint to support the indication of hypercholesterolemia or mixed hyperlipidemia. Indeed, a large body of epidemiological evidence now exists demonstrating a strong positive correlation and causal relationship between serum low density lipoprotein cholesterol LDL-C, and the risk of coronary heart disease (CHD).

It should be recognised that the effect of garlic on LDL-cholesterol is small and the confident interval is rather large ([-11.77; -1.05]). Thus, the size of the effect is modest, and the robustness of the effect is debatable.

Results on HDL cholesterol and triglycerides: Meta-analysis of 30 trials on the effect of garlic on HDL cholesterol levels was significant but small (mean difference HDL = 1.49 [95%CI: 0.19, 2.79] mg/dL, I2 = 33%; P = 0.02). Subgroup analysis of trials using garlic oil revealed the largest and statistically significant effect (mean difference HDLgarlic oil = 5.97 [95%CI: 1.65, 10.30] mg/dL, I2 = 67%; P = 0.007, n = 6), whereas results of subgroup analysis of trials investigating other types of garlic were not significant. The effect of garlic on triglycerides levels was reported by 32 trials but did not reveal a significant effect (mean difference triglycerides = -5.45 [95%CI: -14.18, 3.27] mg/dL, I2 = 71%; P = 0.22).

Assessor's comments
The meta-analysis suggest that garlic could i) reduce total cholesterol to a modest extent (-15.25 mg/dL) ii) reduce LDL cholesterol ((-6.41 mg/dL) iii) increase HDL cholesterol (+1.49 mg/dL). Subgroup analysis by type of product show contradictory results. For example, a greater cholesterol-lowering effect is observed for aged garlic extract without effect on LDL-cholesterol or HDL.

Nevertheless, all these results should be interpreted with caution as heterogeneity is high among all the meta-analysis performed, notably in subgroup analysis of trials of longer duration or with higher baseline cholesterol levels. There is no sensitivity analysis with an evaluation performed with or without studies that were identified as outliers.

Standardised preparations

Mader (1990): In a multicentric placebo-controlled randomised study the effect of standardised garlic-powder tablets (Kwai, Sapec) in the treatment of hyperlipidaemia was investigated. A total of 261 patients of 30 general practitioners in West Germany with total cholesterol and/or triglyceride values more than 200 mg/dl (mostly hyperlipoproteinaemia type II a/II b) took part in the study. Patients were randomly allocated to take tablets containing a total of 800 mg garlic powder (standardised to 1.3% of alliin content) daily or the same number of placebo tablets for 16 weeks (monthly controlled). 221 patients were used for statistical analysis of total cholesterol and 219 patients for the analysis of triglyceride values. Mean serum cholesterol levels dropped in the verum group from 266 to 235 mg/dl (i.e. 12%) during the 4 month treatment period, mean triglyceride values fell in the verum group from 226 to 188 mg/dl (i.e. 17%). The best cholesterol lowering effects were seen in the patients with initial total cholesterol values between 250-300 mg/dl. The difference between the verum and placebo group was highly significant (p less than 0.001).

Assessor's comments
No power calculation has been reported.
The results were not analysed on an intention-to-treat basis. This could inflate the effect size of the intervention.

Neil (1996): This is a randomised, double-blind six-month parallel trial conducted in 115 patients with total cholesterol of 6-8.5 mmol/l and LDL cholesterol of 3.5 mmol/l or above after six weeks of dietary

advice. The active treatment group received dried garlic tablets (standardised to 1.3% allicin) at a dosage of 300 mg three times daily. The control group received a matching placebo. There were no significant differences between the groups receiving garlic and placebo in the mean concentrations of serum lipids (total cholesterol and LDL cholesterol), lipoproteins or apo A1 or B, by analysis either on intention-to-treat or treatment received.

Over the past decade, several intervention studies have investigated the effectiveness and properties of garlic on cholesterol and triglycerides. Among those who showed potential antilipidemic effect since the ESCOP monograph:

Asharf (2005): This 12 week randomised, single-blind, placebo controlled study was conducted on Type 2 diabetic patients with newly diagnosed dyslipidemia (n=70). Patients were divided into two groups each comprising of 35 patients, they were given tablet garlic (Garlex-Bosch Pharmaceuticals) 300 mg (containing 1.3% allicin) twice daily and identical placebo tablets respectively. After 12 weeks the garlic treated group (n = 33) had a significant reduction in total cholesterol (-28 mg/dl, - 12.03% P= <0.001), LDL - C (-30 mg/dl, - 17.99% P=<0.001) while the placebo treated group (n=32) had a non significant decrease in total cholesterol (- 2 mg/dl, - 0.9% p= ns) and LDL-C (-3 mg/dl, -1.6% p=ns). HDL cholesterol was significantly increased in patients treated with garlic (3.35 mg/dl, 8.81% P= <0.05) compared with placebo group (0.62, 1.6% P= n.s) but there was no significant difference in triglyceride was observed between two groups.

Sobenin (2008): The lipid-lowering effects of time-released garlic powder tablets, Allicor (600 mg daily), were investigated in a double-blinded placebo-controlled randomised study in 42 men aged 35-70 with mild hypercholesterolemia. After 12 weeks of treatment, total cholesterol in Allicor-treated patients had fallen by 7.6% (p=0.004) as compared to the level at randomisation, and was 11.5% lower than the placebo group (p=0.005). LDL cholesterol in Allicor-treated patients fell by 11.8% (p=0.002) and 13.8% (p=0.009), respectively. HDL cholesterol also increased significantly after 8 and 12 weeks of treatment. By the end of the study, HDL cholesterol in Allicor-treated patients had increased by 11.5% (p=0.013).

Sobenin (2010): The double-blinded placebo-controlled randomised study has been performed in 51 coronary heart disease (CHD) patients who had a serum cholesterol level above 200 mg/dl (5.2 mmol/L) to estimate the effects of time-released garlic powder tablets Allicor on the values of 10-year prognostic risk of acute myocardial infarction (fatal and non-fatal) and sudden death, with the respect of secondary CHD prevention. In the placebo group, no statistically significant changes were observed in lipids levels, except to triglycerides that lowered by 16.4% (95% CI: 5.1; 36.6 mg/dl, p = 0.004) from the baseline. In Allicor-treated patients there were significant changes in total and LDL cholesterol levels. Total cholesterol decreased by 12.4% (95% CI: 12.3; 54.8 mg/dl, p = 0.004), and LDL cholesterol decreased by 16.3% (95% CI: 14.8; 45.8 mg/dl, p = 0.001) from the baseline.

Others trials failed to show such effect or showed mixed results since ESCOP monograph:

Peleg (2003): This was a randomised, double-blind, placebo-controlled, 16 weeks, parallel treatment including 33 patients with primary hypercholesterolemia. Garlic in the form of alliin 22.4 mg/day (garlic powder) was given to 13 patients, and placebo to 20. No significant changes were observed in levels of total cholesterol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and triglycerides.

Satitvipawee (2003): The authors performed a randomised, double-blind, 12 weeks, placebo-controlled trial in 136 hypercholesterolemic subjects (cholesterol concentrations ≥ 5.2 mmol/L). The subjects were randomly assigned to receive an enteric-coated Thai garlic extract tablet once daily (standardised to 1.12% allicin or 5.6 mg/tablet) or placebo. There were no statistically significant changes in serum

total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol after the 12-week treatment.

Tanamai (2004): The present study aimed at investigating the cholesterol-lowering and side effects of garlic enteric coated tablets in comparison with placebo tablets. The study is a randomised double-blinded crossover design involving 116 volunteers. However, 16 of them did not complete the study. The remaining 100 volunteers were divided into two groups: 45 were in the trial group and the remaining 55 in the control group. The volunteers in the trial group were asked to take garlic tablets in the first three months, placebo in the second three months and discontinue all tablets in the last three months, while the volunteers in the control group started with three months of placebo followed by three months of garlic tablets and ended up with three months of tablets discontinuity. The results showed that there were no significant differences in the total serum cholesterol levels between the two groups at the end of three months or six months of the study.

Turner (2004): This was a randomised, double-blind, placebo-controlled, 12 weeks, parallel treatment including 75 healthy, normo-lipidaemic volunteers assigned to dried garlic powder tablets (10.8 mg alliin (3-(2-propenylsulfinyl)-L-alanine)/d, corresponding to about three garlic cloves) or placebo. No significant differences between the garlic and placebo groups were detected for serum total cholesterol concentration, LDL-cholesterol, HDL-cholesterol.

Van Doorn (2006): This was a double-blind, randomised, placebo-controlled trial in 90 overweight [body mass index (in kg/m2) > 24.5] subjects aged 40-75 y who smoked >10 cigarettes/d. The subjects were randomly assigned to 3 parallel treatment groups: garlic powder (2.1 g/d), atorvastatin (40 mg/d), or placebo. No significant differences between the garlic-treated and the placebo groups are observed on lipids parameters. In contrast, compared with the placebo group, atorvastatin treatment resulted in significantly lower plasma concentrations of C-reactive protein (20.2%; 1.7%, 35.3%), total cholesterol (37.2%; 33.1%, 41.1%), LDL cholesterol (52.7%; 47.9%, 57.1%), triacylglycerols (31.9%; 20.8%, 41.5%).

Assessor's conclusions on clinical trials published since the ESCOP monograph
The majority of clinical trials published since the ESCOP monograph suggested that garlic did not produce any statistically significant reduction in total cholesterol and triglycerides. More importantly, results on LDL cholesterol are inconclusive. Therefore, considering the conflicting results of clinical trials, the lake of data regarding the long term maintaining effet, no well established use could be granted in hypercholesteroleamia (primary and mixed).

Conclusion on the garlic effect on antilipidemic effects:

The effect of garlic on cholesterol or other lipid parameters has been investigated in numerous trials and meta-analyses, with variable results. Although trials conducted before 1995 suggest a beneficial effect of garlic on lipid concentration, more recent reviews and trials, published between 2002 and 2012, reported a more modest role for garlic on plasma lipid levels or no effects.

The diverse composition and amount of active sulfur compounds of different garlic preparations used in various trials might be responsible for the above mentioned inconsistent findings.

Other factors like subject recruitment, duration of study, dietary control, lifestyle, and methods of lipid analyses may also have an influence. These findings emphasise the need for standardisation of garlic preparations in order to reach to a valid conclusion.

Regarding this last point, a recent study (Gardner, 2007) comparing the effects of raw garlic, powdered garlic supplement and aged garlic extract versus placebo, is unable to show a clinically relevant effect of garlic on plasma lipid concentrations.

When alliin contains is considered, results from clinical trials with doses of alliin showed significant showed conflicting results with impossibility to draw any conclusion.

Clinically, the lipid lowering effect of garlic observed here is far less than prescription products such as statins, fibrates or niacin. Long-term effects on lipids or cardiovascular morbidity and mortality remain unknown.

Inconsistent clinical evidence warrants more study before reaching convincing conclusions. Thus, data are insufficient to grant an indication for hypercholesterolemia.

b. Antihypertensive effects

Hypertension is considered as major risk factor for several cardiovascular and related diseases as well as for diseases leading to a marked increase in cardiovascular risk (Mancia G, 2007).

The combination of this condition and the large prevalence of high blood pressure in the population (Kearney PM, 2005; Martiniuk AL, 2007; Wolf-Maier K, 2003) leads the World Health Organisation to consider high blood pressure as the first global risk for mortality in the world (responsible for 13% of deaths globally) (Global health risks, WHO 2009).

Mortality related to ischemic heart disease or to stroke increases progressively and linearly with blood pressure increase. Throughout middle age (i.e., at ages 40–69 years), each 20 mmHg SBP or 10 mmHg DBP increase is associated with more than a 2-fold difference in the stroke death rate, and with 2 fold differences in the death rates from ischemic heart disease (Lewington S, 2002).

Standardised preparations

The WHO monograph: WHO monograph has held a meta-analysis from Silagy et al., 1994. This meta-analysis of the effect of Bulbus Allii Sativi on blood pressure reviewed a total of 11 randomised, controlled trials (published and unpublished). A total of 11 randomised controlled trials that included an examination of the effect of garlic on blood pressure were identified, and 8 were eligible for inclusion (which provided data on 415 subjects). The median duration of the trials was 12 weeks. All these studies have utilised the same garlic powder preparation (tablets) at a dose of 600– 900mg daily. Only three of the trials specifically used hypertensive subjects, and many of the studies suffered from methodological flaws. The mean difference in reduction of mean systolic blood pressure between garlic-treated and placebo- treated subjects was -7.7 mmHg (95% Confidence Intervals -4.3, -11.0 mmHg), and for diastolic blood pressure was -5.0 mmHg (95% CI-2.9,-7.1 mmHg).

Who monograph conclude that the results of the meta-analysis led to the conclusion that garlic may have some clinical usefulness in mild hypertension, but there is still insufficient evidence to recommend the drug as a routine clinical therapy for the treatment of hypertension.

ESCOP monograph: ESCOP monograph cites 8 trials using garlic powder at a dose of 600-900 mg daily in patients with hypercholesterolaemia, with mild or moderate hypertension, or normotensive patients showing various reductions of systolic blood pressure (SBP) and diastolic blood pressure (DBP) from no reduction to 19% and no reduction from 17% respectively.

Meta-analysis

Ried K (2008): The most recent meta-analysis has been published by Ried K et al., 2008 (BMC Cardiovasc Disord. 2008)

Methods
The authors have searched the Medline and Embase databases for studies published between 1955 and October 2007. Randomised controlled trials with true placebo groups, using garlic-only preparations,
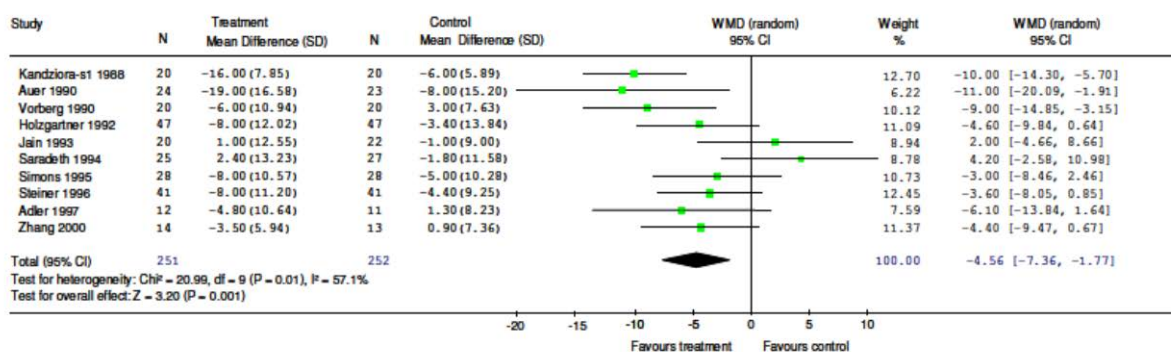
and reporting mean systolic and/or diastolic blood pressure (SBP/DBP) and standard deviations were included in the meta-analysis. They also conducted subgroup meta-analysis by baseline blood pressure (hypertensive/normotensive), for the first time. Meta-regression analysis was performed to test the associations between blood pressure outcomes and duration of treatment, dosage, and blood pressure at start of treatment.
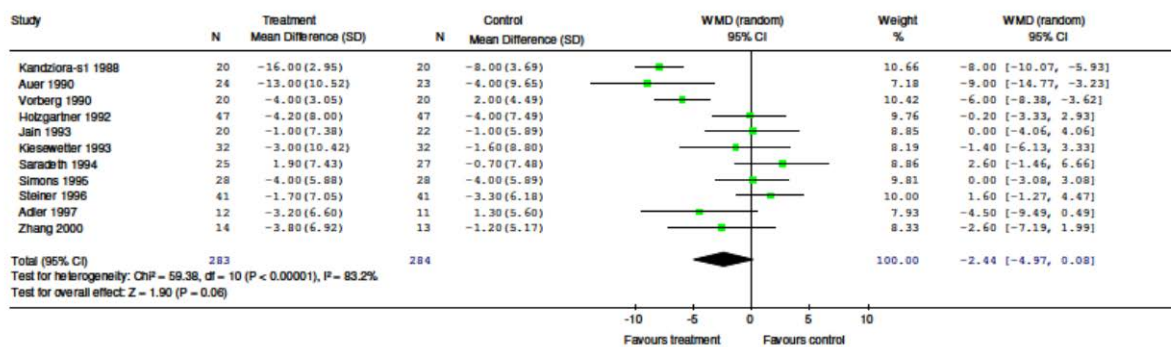
Results

Eleven of 25 studies included in the systematic review were suitable for meta-analysis. Nine studies used garlic powder (at a dose of 600-900 mg/day), one study used aged garlic extract and another used distilled garlic oil. Meta-analysis of all studies showed a mean decrease of 4.6 ± 2.8 mm Hg for SBP in the garlic group compared to placebo (n = 10; p = 0.001), while the mean decrease in the hypertensive subgroup (mean SBP> 140 mmHg or mean DBP> 90 mm Hg) was 8.4 ± 2.8 mm Hg for SBP (n = 4; p < 0.001), and 7.3 ± 1.5 mm Hg for DBP (n = 3; p < 0.001):

Table 11: Meta-analysis graphs on the effect of garlic on systolic blood pressure or diastolic blood pressure.

A) SBP all studies

Study	N	Treatment Mean Difference (SD)	N	Control Mean Difference (SD)	WMD (random) 95% CI	Weight %	WMD (random) 95% CI
Kandziora-s1 1988	20	−16.00 (7.85)	20	−6.00 (5.89)		12.70	−10.00 [−14.30, −5.70]
Auer 1990	24	−19.00 (16.58)	23	−8.00 (15.20)		6.22	−11.00 [−20.09, −1.91]
Vorberg 1990	20	−6.00 (10.94)	20	3.00 (7.63)		10.12	−9.00 [−14.85, −3.15]
Holzgartner 1992	47	−8.00 (12.02)	47	−3.40 (13.84)		11.09	−4.60 [−9.84, 0.64]
Jain 1993	20	1.00 (12.55)	22	−1.00 (9.00)		8.94	2.00 [−4.66, 8.66]
Saradeth 1994	25	2.40 (13.23)	27	−1.80 (11.58)		8.78	4.20 [−2.58, 10.98]
Simons 1995	28	−8.00 (10.57)	28	−5.00 (10.28)		10.73	−3.00 [−8.46, 2.46]
Steiner 1996	41	−8.00 (11.20)	41	−4.40 (9.25)		12.45	−3.60 [−8.05, 0.85]
Adler 1997	12	−4.80 (10.64)	11	1.30 (8.23)		7.59	−6.10 [−13.84, 1.64]
Zhang 2000	14	−3.50 (5.94)	13	0.90 (7.36)		11.37	−4.40 [−9.47, 0.67]
Total (95% CI)	251		252			100.00	−4.56 [−7.36, −1.77]

Test for heterogeneity: Chi² = 20.99, df = 9 (P = 0.01), I² = 57.1%
Test for overall effect: Z = 3.20 (P = 0.001)

-20 -15 -10 -5 0 5 10
Favours treatment   Favours control

B) DBP all studies

Study	N	Treatment Mean Difference (SD)	N	Control Mean Difference (SD)	WMD (random) 95% CI	Weight %	WMD (random) 95% CI
Kandziora-s1 1988	20	−16.00 (2.95)	20	−8.00 (3.69)		10.66	−8.00 [−10.07, −5.93]
Auer 1990	24	−13.00 (10.52)	23	−4.00 (9.65)		7.18	−9.00 [−14.77, −3.23]
Vorberg 1990	20	−4.00 (3.05)	20	2.00 (4.49)		10.42	−6.00 [−8.38, −3.62]
Holzgartner 1992	47	−4.20 (8.00)	47	−4.00 (7.49)		9.76	−0.20 [−3.33, 2.93]
Jain 1993	20	−1.00 (7.38)	22	−1.00 (5.89)		8.85	0.00 [−4.06, 4.06]
Kiesewetter 1993	32	−3.00 (10.42)	32	−1.60 (8.80)		8.19	−1.40 [−6.13, 3.33]
Saradeth 1994	25	1.90 (7.43)	27	−0.70 (7.48)		8.86	2.60 [−1.46, 6.66]
Simons 1995	28	−4.00 (5.88)	28	−4.00 (5.89)		9.81	0.00 [−3.08, 3.08]
Steiner 1996	41	−1.70 (7.05)	41	−3.30 (6.18)		10.00	1.60 [−1.27, 4.47]
Adler 1997	12	−3.20 (6.60)	11	1.30 (5.60)		7.93	−4.50 [−9.49, 0.49]
Zhang 2000	14	−3.80 (6.92)	13	−1.20 (5.17)		8.33	−2.60 [−7.19, 1.99]
Total (95% CI)	283		284			100.00	−2.44 [−4.97, 0.08]

Test for heterogeneity: Chi² = 59.38, df = 10 (P < 0.00001), I² = 83.2%
Test for overall effect: Z = 1.90 (P = 0.06)

-10 -5 0 5 10
Favours treatment   Favours control

Sub-group meta-analysis of "normotensive" individuals was not significant. Regression analysis revealed a significant association between blood pressure at the start of the intervention and the level of blood pressure reduction (SBP: R = 0.057; p = 0.03; DBP: R = -0.315; p = 0.02).

Heterogeneity was moderate (I2=57.1%) of SBP. The same trend was observed for DBP (I2=83.2%).

Regression analysis was conducted to test whether heterogeneity between the studies could be explained by one or more of the following continuous variables: dosage (only studies using garlic powder were included, n = 8/9 (SBP/DBP), range 600–900 mg/d), duration of intervention (SBP/DBP: n = 10/11, range 12–23 wks), and SBP or DBP at start of intervention (SBP/DBP: n = 10/11, range 175–109 SBP/102-64 DBP). SBP or DBP at start of intervention proved to be a significant predictor for heterogeneity (SBP: R = -0.151, p = 0.03; DBP: R = -0.316, p = 0.02), strengthening the results of subgroup meta-analysis.

None of the other variables tested showed a significant association with blood pressure outcomes (data not shown).

Assessor's comments

Sensitive analysis shows no difference on the results regarding the type of garlic preparation.

This meta-analysis has been reviewed by Simons et al., 2009. The authors have made a review on the influence of trial quality on the effect of garlic on blood pressure. The authors conclude that the methodological quality of the studies was poor. Indeed, methodological quality was assessed using a scoring card derived from the Cochrane checklist "the assessment of a randomised trial". The card consisted of nine items: Allocation concealment (AC), randomisation, patients blinded (PB), researchers blinded, evaluators blinded (EB), comparable groups (CG), adequate lost-to-follow up analysis, intention-to-treat analysis (IT) and groups receiving same treatment (ST).

Regarding the quality analysis of the trials included by Ried *et al.*, the results are the following:

Table 12: Quality analysis of the trials included in the meta-analyses performed by Ried *et al.,* or by Silagy and Neil

Table 5. *Quality analysis of the 14 included trials in the meta-analyses performed by Ried et al.[6] or by Silagy and Neil[5]*

Author (year) [reference]	Included by Ried (R) or Silagy (S)	Total points quality scale (max. 9)	Lacking items	Total points blood pressure scale (max. 5)	Lacking items
Adler (1997)[15]	R	4	AC, PB, EB, CG, IT	1	DM, AH, RP, NR
Auer (1990)[16]	R + S	4	AC, EB, CG, D, IT	1	DM, AH, RP, NR
De A Santos (1993)[21]	S	6	AC, EB, IT	0	DM, AH, BP, RP, NR,
Holzgartner (1992)[27]	R + S	5	AC, EB, IT, ST	0	DM, AH, BP, RP, NR
Jain (1993)[30]	R + S	5	AC, EB, D, IT	4	AH
Kandziora (1988)[31]	S	4	AC, PB, EB, D, ITT	2	DM, AH, RP
Kandziora (1988)[32]	R + S	4	AC, EB, D, IT	2	DM, AH, RP
Kiesewetter* (1991)[47]	S	4	AC, EB, CG, D, IT	0	DM, AH, BP, RP, NR
Kiesewetter (1993)[33]	R	5	AC, EB, D, IT	2	DM, AH, NR
Saradeth (1994)[39]	R	4	AC, EB, CG, D, IT	0	DM, AH, BP, RP, NR
Simons (1995)[40]	R	5	AC, EB, CG, IT	4	AH
Steiner (1996)[41]	R	4	AC, EB, CG, D, IT	1	BP, AH, RP, NR
Vorberg (1990)[44]	R + S	5	AC, EB, CG, IT	1	DM, AH, RP, NR
Zhang (2000)[45]	R	6	AC, EB, D, IT	3	BP, AH

AC = allocation concealment; PB = patient blinding; EB = evaluators blinding; D = dropouts; CG = comparable groups; IT = intention-to-treat analysis; ST = same treatment of groups; DM = device mentioned; AH = arm at heart level; BP = body position; RP = resting period; NR = number of reading. *Not included in our systematic review because of a treatment period of less than eight weeks.

Inclusion criteria of Ried work are only blinding, randomisation and blood pressure as a primary outcome. Simons et al. shows that what trials mostly lack is adequate allocation concealment, blinding of the evaluators and proper use of an intention-to-treat analysis.

In addition, they conclude that trials provide insufficient information about the technique used to measure blood pressure.

Assessor's comments

Agree with analysis of Simmons et al. The absence of a proper methodology may have indeed affected outcome.

Moreover, Simmons et al. have made an analysis based on the five clinical trials with sufficient quality data based on the criteria put forward:

Table 13: Results of the five clinical trials with the highest methodological quality score

Author (year) [reference]	Blood pressure a primary goal	Hypertensive population	Methodological quality[*]	Blood pressure quality[**]	Garlic effective in lowering blood pressure
Gardner (2001)[25]	No	No	7	4	No
Jain (1993)[30]	No	No	5	4	No
Simons (1995)[40]	No	No	5	4	No
Turner (2004)[43]	Yes	No	8	4	No
Zhang (2000)[45]	Yes	No	6	3	No

Table 6. Summary of the five clinical trials on the effect of garlic on blood pressure with the highest methodological quality score and highest score for the quality in reporting blood pressure measurements

[*]Score based on the criteria proposed by the Dutch Cochrane society. No points indicates a high suspicion of bias. A cutoff value of five points or more was chosen. [**]Blood pressure quality was scored on reporting on exact device, body position, arm at heart level, resting period and number of readings reported. A maximum of five point could be obtained. A cutoff value of three points was chosen.

Assessor's comments

These studies do not show an effect of garlic on blood pressure. Nevertheless, all these trials have been conducted on normotensive subjects.

Stabler (2012): This Cochrane meta-analysis has been recently published in order to determine whether the use of garlic as monotherapy, in hypertensive patients, lowers the risk of cardiovascular morbidity and mortality compared to placebo.

The search identified two randomised controlled trials for inclusion. One trial (Auer et al., 1990) included 47 hypertensive patients and showed that garlic significantly reduces mean supine systolic blood pressure by 12 mmHg (95% CI 0.56 to 23.44 mmHg, p=0.04) and mean supine diastolic blood pressure by 9 mmHg (95% CI 2.49 to 15.51 mmHg, p=0.007) versus placebo. The authors state that garlic was "free from side effects" and that no serious side effects were reported. There were 3 cases "where a slight smell of garlic was noted."

The second trial (Kandziora et al., 1988) could not be meta-analysed as they did not report the number of people randomised to each treatment group. They did report that 200 mg of garlic powder given three times daily, in addition to hydrochlorothiazide-triamterene baseline therapy, produced a mean reduction of systolic blood pressure by 10-11 mmHg and of diastolic blood pressure by 6-8 mmHg versus placebo.

Neither trial reported clinical outcomes and insufficient data were provided on adverse events.

The authors conclude that the evidence currently available is insufficient to determine whether garlic provides a therapeutic advantage versus placebo in terms of reducing the risk of cardiovascular morbidity and mortality.

Clinical trials of interest

Ried (2010): The aim of this trial is to assess the effect, tolerability and acceptability of aged garlic extract as an adjunct treatment to existing antihypertensive medication in patients with treated, but uncontrolled, hypertension.

This is a double-blind parallel randomised placebo-controlled trial involving 50 patients whose routine clinical records in general practice documented treated but uncontrolled hypertension. The active treatment group received four capsules of aged garlic extract (960 mg containing 2.4 mg S-allylcysteine) daily for 12 weeks, and the control group received matching placebos. The primary outcome measures were systolic and diastolic blood pressure at baseline, 4, 8 and 12 weeks, and change over time.

Assessor's comments

When the blood pressure has been measured under trial condition, among the 50 patients included,

only 40% of participants have a mean SBP > 140 mmHg at baseline. Thus, the recruitment of patients is questionable.

The baseline characteristics between both groups are very different: patients in the control group have a higher SBP (151 versus 146), are more current smokers (12% versus 4%) and diabetics (32% versus 20%).

Moreover, the use of other anti-hypertensive agents was not standardized or equal between groups.

The results show the intention-to-treat analyses of SBP including all participants did not reveal a significant difference between the groups from baseline to 12 weeks. In patients with uncontrolled hypertension (SBP ≥ 140 mmHg at baseline), systolic blood pressure was on average 10.2 ± 4.3 mmHg (p=0.03) lower in the garlic group compared with controls over the 12-week treatment period. Changes in blood pressure between the groups were not significant in patients with SBP<140 mmHg at baseline.

Assessor's comments

The results obtained on the subgroup of only 20 patients with SBP > 140 mm Hg are not meaningful and should be considered exploratory.

Ried (2013): This is a double-blind, randomised, 12 weeks, placebo-controlled trial including 79 patients with uncontrolled hypertension (SBP>140 mmHg as recorded on their medical records in the past 6 months). Participants were allocated to one of three garlic groups with either of one, two or four capsules daily of aged garlic extract (240/480/960mg containing 0.6/1.2/2.4mg of S-allylcysteine) or placebo. Mean systolic blood pressure was significantly reduced by 11.8±5.4mmHg in the garlic-2-capsule group over 12 weeks compared with placebo (P=0.006), and reached borderline significant reduction in the garlic-4-capsule group at 8 weeks (-7.4±4.1mmHg, P=0.07). Changes in systolic blood pressure in the garlic-1-capsule group and diastolic blood pressure were not significantly different to placebo.
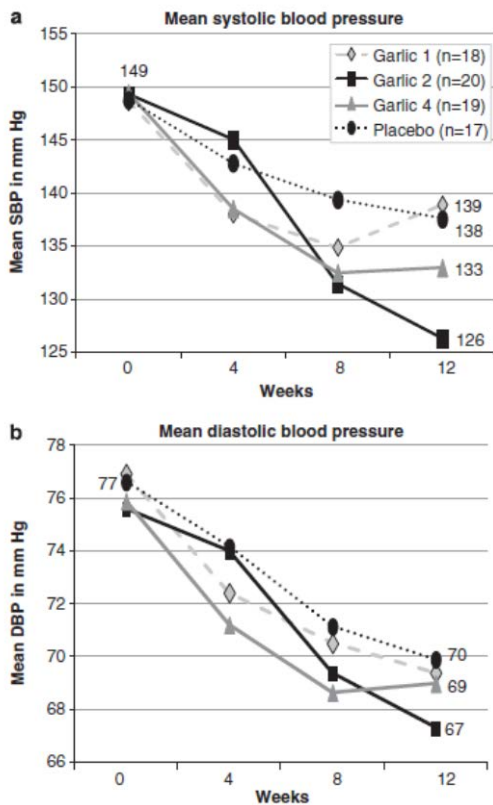

Figure 5: Effects of aged garlic extract on blood pressure in Ried trial (2013)

Assessor's comments

The number of patients per group is low. Baseline characteristics and antihypertensive drugs differ between the different groups. This trial suggests that aged garlic extract at 480 mg (containing 1.2 mg of S-allylcysteine) but not at 960 mg could decrease systolic blood pressure.

Sobenin (2009): a double-blind, randomised, 8 weeks, placebo-controlled trial conducted in 84 men with newly diagnosed mild and moderate hypertension (SBP between 150 and 160 mmHg; DBP between 90 ans 115 mmHg). Patients were randomised to receive daily for 8 weeks: Allicor 600 mg (n=30) (one tablet containing 300 mg garlic powder twice); placebo (n = 20) ; Allicor 2400 mg (n=18) ; and Kwai 900 mg (one tablet containing 300 mg garlic powder three times a day) (n=16). Arterial blood pressure was measured (in the morning on the right and left arms, in supine, sitting and standing positions) at the time of inclusion and every 4 weeks thereafter for 16 weeks of the trial. The results of the second and the third measurements were recorded, and the mean value of a total of 12 measurements was used as an integral estimate of arterial blood pressure. SBP in the total group was lowered by 3.2±0.7 mm Hg (95% CI: 1.7–4.6, Po0.001). After 8 weeks of treatment, SBP was lowered significantly compared to placebo by 7.0±0.8 mm Hg (95% CI: 5.3–8.7, P<0.001) in 600 mg Allicor daily group, 9.3±0.7 mm Hg (95% CI: 7.8–10.9, P<0.001) 2400 mg Allicor daily group, 5.4±1.6 mm Hg (95% CI: 1.9–8.8, P<0.010) in 900 mg Kwai daily group.

Assessor's comments

This trial showed a blood pressure lowering effect of garlic powders tablets with no significant additionnel dose effet on antihypertensive. However, considering low number of patients included, and the short treatment duration, the results of this trial are insufficient to support antihypertensive indication

Conclusion on potential antihypertensive effects of garlic

The effects of garlic on blood pressure cannot be ascertained. Meta-analysis and clinical trials have been performed (Ried *et al.*, 2008, updated on 2016; Rohnel *et al.*, 2015; Wang *et al.*, 2015). The results suggest that garlic preparations could reduce blood pressure in individuals with hypertension. Nevertheless, the trials on the effects of garlic on blood pressure suffer of inadequate study designs, low number of patients included, short duration and methodological deficiencies. Indeed, in the last meta-analysis, only seven randomised controlled trials were retrieved, just 3 were conducted in a double blind manner, and no has exceeded 12 weeks treatment duration. Furthermore, alliin containing was not specified.

Thus, considering the fact that the use of garlic preparation in hypertension management is insufficiently studied with no clinical data on the long term effet persistence, it can not be recommended as an interventionnel advice for hypertensive patients in daily practice, and a well established use in this indication could not be granted.

c. *Antithrombotic effects/Cardiovascular morbidity and mortality*

Allicin and allicin-derived thiosulfinates are considered as major compounds responsible for the antithrombotic activity of garlic (Cavagnaro PF, 2007).

Platelet aggregation inhibition

Standardised preparations

A review regarding effects of garlic on platelet (Rahman K, 2007) reported that various mechanisms are involved in this activity: inhibition of cyclooxygenase activity, suppression of platelet intracellular calcium mobilisation, inhibiting cyclooxygenase activity, increasing cAMP levels, increasing availability of platelet NO, and interacting directly with the fibrinogen receptor GPIIb/IIIa and inhibiting its

exposure to fibrinogen. However, inhibiting cyclooxygenase activity and direct interaction with fibrinogen were reported as the main mechanisms for garlic platelet inhibition effect (Chang HS, 2005; Ali M, 1995).

Clinical trial of interest: Several clinical trials have investigated the potential antiplatelet effect of garlic:

Steiner M, 2001: In this randomised, double-blind study of healthy volunteers (n = 34), the effect of aged garlic extract (AGE) was evaluated in doses between 2.4 and 7.2 g/d vs. equal amounts of placebo. Platelet aggregation and adhesion were measured at 2 weeks intervals throughout the study. Threshold concentrations for epinephrine and collagen increased moderately during AGE administration compared with the placebo and baseline periods. Only at the highest supplementation level did AGE show a slight increase in the threshold level of ADP-induced aggregation. Platelet adhesion to collagen, fibrinogen and von Willebrand factor was investigated by perfusing whole blood through a laminar flow chamber under controlled flow conditions. Adherence of platelets was inhibited by AGE in a dose-dependent manner when collagen was the adhesive surface perfused at low shear rates (approximately 30 s(-1)). At high shear rates (1200 s(-1)), AGE also inhibited platelet adhesion to collagen but only at higher intake levels. Adhesion to von Willebrand factor was reduced only at 7.2 g/d AGE, but adherence to fibrinogen was potently inhibited at all levels of supplementation.

Kiesewetter H, 1993: In this double-blind, placebo-controlled study on 60 voluntary subjects with cerebrovascular risk factors and constantly increased platelet aggregation, it was demonstrated that the daily ingestion of 800 mg of powdered garlic (in the form of coated tablets) over 4 weeks led to a significant inhibition of the pathologically increased ratio of circulating platelet aggregates and of spontaneous platelet aggregation. The ratio of circulating platelet aggregates decreased by 10.3%, from 1.17 +/- 0.08 to 1.05 +/- 0.11 (P < 0.01), and spontaneous platelet aggregation by 56.3%, from 40.7 +/- 23.3 to 17.8 +/- 23.2 degrees (P < 0.01) during the garlic phase. There were no significant changes in the placebo group. The parallel group comparison (garlic versus placebo) revealed a significantly different ratio of circulating platelet aggregates after 4 weeks of treatment (P < 0.05). After the 4-week wash-out phase the values increased again to 1.19 +/- 0.32 and 34.9 +/- 28.7 degrees, reaching the initial values (run-in phase prior to the ingestion of garlic).

Kiesewetter H, 1993: In this 12-week therapy with garlic powder (daily dose, 800 mg) study in patients with peripheral arterial occlusive disease stage II, thrombocyte aggregation decreased significantly.

Steiner M, 1998: Platelet functions were assessed during the course of an intervention 10 month study comparing the effect of aged garlic extract with placebo on the lipid profiles of moderately hypercholesterolemic men. 7.2 aged garlic extract supplementation per day showed a significant reduction of epinephrine- and, to a lesser degree, collagen-induced platelet aggregation but failed to demonstrate an inhibition of adenosine diphosphate (ADP)-induced aggregation. Platelet adhesion to fibrinogen, measured in a laminar flow chamber at moderately high shear rate, was reduced by approximately 30% in subjects taking AGE compared with placebo supplement.

Beckert BW, 2007: In this blinded study, five commercially available herbal agents were investigated, including garlic. One of the agents was administered to 10 adult volunteers at the manufacturer's recommended dose for 2 weeks. At the end of the 2-week period, *in vivo* platelet function was quantified using the PFA-100 assay. After a 2-week "washout" period, the protocol was repeated using a different agent. This 4-week cycle was repeated for each of the five herbal agents, as well as the control agent aspirin. *In vivo* platelet function was not affected by the administration of any herbal agent and was markedly inhibited with the administration of aspirin.

Assessor's comments
Garlic dosing was not specified. It was only reported that the subjects were administered manufacturer's recommended dose.

Ziaei S, 2001: Effect on platelet aggregation was assessed during this randomised, single-blind, placebo-controlled study. 100 nulliparous pregnant women were treated with daily doses of 800mg garlic powder /day (n=50) or 800mg/day placebo (n=50) during the third trimester of pregnancy. The inhibition of platelet aggregation did not show any significant difference before and during the treatment.

Non Standardised preparations

Wojcikowski K, 2007: In this randomised, double-blind, placebo-controlled, crossover study, acute effect of garlic on platelet aggregation was tested in 14 healthy volunteers. The active agent tested was solvent-extracted garlic oil incubated in ethanol. Platelet aggregation was induced *ex vivo* by adrenaline, collagen or adenosine diphosphate (ADP). Four hours after consuming one large dose of oil derived from 9.9 g garlic, there was little or no effect in the reduction of platelet aggregation. Platelet aggregation induced by adrenaline was reduced slightly but significantly ($P<0.05$; 12% reduction). The oil had no effect on collagen- or ADP-induced aggregation.

Barrie SA, 1987: In this double-blind, two period, cross-over trial the effect of oral ingestion of garlic oil was studied in 20 healthy volunteers. The subjects were randomised into 2 groups. Each group rotated for four week periods through 2 different sequences of oral supplementation including: 18 mg of garlic oil (extracted from 9 grams of fresh garlic) and placebo. The amount of platelet aggregation decreased significantly ($p< .005$) during garlic administration.

Dietary garlic

Scharbert G, 2007: In this randomised, crossover, observer-blinded, placebo-controlled study, whole blood from 18 healthy volunteers was investigated before and 5 h after ingestion of the study medication consisting of Greek tsatsiki with 4.2 g raw garlic (verum), or Greek tsatsiki without garlic (placebo),. The potential long-term effects of garlic were investigated in five volunteers after daily ingestion of 4.2 g of raw garlic over 1 week. Platelet function was assessed with the PFA-100 assay, impedance aggregometry, and thrombelastographic Platelet Mapping. *In vitro* experiments were performed to prove the sensitivity of the assays to garlic-induced platelet inhibition.

Baseline values of platelet function were within normal range in all volunteers. Platelet function was not impaired by single and repeated oral consumption of Greek tsatsiki containing raw garlic in any point-of-care monitoring test used.

Ali M, 1995: In this open study 8 healthy volunteers took 1 clove of garlic (about 3 g) daily for 16 weeks. Serum thromboxane B_2 was reduced from 243 to 24 ng/ml ($p<0.001$) (Ali M, 1995)

Assessor's comments
Although the in vivo anti platelet seems well established, the mixed data issued from randomized controlled clinical studies, quality weakness of some of them does not allow concluding on the anti platelet effect of garlic. Moreover, even if an antiplatelet effect is considered, the clinical relevance of such effect should be assessed through morbimortality clinical trials (secondary prevention in patients with coronary heart disease, previous stroke, at high CV risk etc.)

Fibrinolytic activity

The data regarding fibrinolytic activity suggests mixed effect and are mostly issued from early studies. fibrinolytic activity was reported in at least 9 studies (Gadkari JV, 1991; Bordia A, 1998; Bordia AK, 1978; Bordia AK, 1978; Chutani SK, 1988; Arora RC, 1981 ; Bordia AK, 1982; Legnani C, 1993;

Luley C, 1986; Chutani SK, 1981) most of them conducted with essential garlic oil. 2 other studies showed no effect on fibrinolytic activity (Arora RC, 1981; Lutomski J.; 1984).

Assessor's comments
The available data suggest a limited effect on increasing fibrinolytic activity, but no conclusion can be made. Therapeutic usefulness of such effect should be investigated through clinical trials with relevant clinical endpoints.

Effect on plasma viscosity

Standardised preparations

Clinical trials: only one clinical trial of interest was reviewed :

Kiesewetter (1993): In a double blind study (Kiesewetter H, 1993) 80 patients with peripheral arterial occlusive disease received 800 mg daily of standardised garlic powder or placebo for 12 weeks. In the garlic group plasma viscosity decreased by 3.6% (p<0.0013).

Decrease of plasma viscosity was reported after administration of garlic powder (Kwai, Sapec; total dose of 900 mg garlic powder) in a randomised placebo-controlled double-blind cross-over study conducted in healthy volunteers (Jung EM, 1991).

Assessor's comments
The available data are limited and suggest a slight effect on decreasing plasma viscosity, but no conclusion can be made. Therapeutic usefulness of such effect should be investigated through clinical trials with appropriate clinical endpoints.

Vascular effect

Standardised preparations

Clinical trials: only one clinical trial of interest was reviewed:

Breithaupt-Grögler (1997): In an open study involving 101 healthy volunteers, the effects of taking 300-900 mg of standardised garlic powder (1.3% alliin) daily on elastic properties of the aorta were investigated for at least 2 years. Compared to results from an untreated control group, garlic intake attenuated age-related increases in aortic stiffness: significant differences were observed in pulse wave velocity (8.3 vs. 9.8 m/s, p<0.0001) and pressure-standardise elastic vascular resistance (0.63 vs. 0.9 m^2/s^2 x mm Hg, p<0.0001); correlation with age was significantly different (p<0.0001) for pulse wave velocity (garlic, r = 0.44; control, r = 0.52) and systolic blood pressure (garlic, r = 0.48; control, r = 0.54) ().

Assessor's comments
These data support the hypothesis that garlic intake had a protective effect on the elastic properties of the aorta related to aging in humans. However, only clinical trials with strong clinical endpoints can demonstrate a clinical benefit.

Effects on Cardiovascular Morbidity and Mortality

Standardised preparations

Only three clinical trials with cardiovascular outcomes were published. Ackermann RT *et al.,* reviewed these clinical trials and reported the following comments:

Peripheral arterial occlusive disease: Two trials assessed improvement in pain-free walking distance in subjects with lower extremity peripheral vascular disease treated with garlic vs placebo (Kiesewetter H, 1993; Czerny B, 1996). Although the authors of one trial reported significant increases in the walking

distance with standardised dehydrated tablets (Kwai), there was a 20% dropout rate with no intention-to-treat analysis, significant disparities in the reporting of a garlic taste between garlic-treated and placebo groups that suggested possible inadequate blinding, and a reanalysis using baseline walking distances from the time of randomisation rather than during a run-in phase no longer yielded significant results (Kiesewetter H, 1993). The second trial reported statistically significant increases in pain-free walking but used a garlic oil macerate–soya lecithin–hawthorn oil–wheat germ oil combination, making it difficult to assess the independent effect of garlic on this end point (Czerny B, 1996)"

Coronary heart disease: One 3-year trial assessing reinfarction rates in 432 patients with evidence of a prior myocardial infarction reported 11 deaths and 15 reinfarctions in 222 subjects randomised to a garlic extract (0.1 g/kg per day for body mass) and 20 deaths and 22 reinfarctions in the 210 placebo recipients (Bordia A, 1989). Although the author reported significant differences, reanalysis of between-group comparisons using a χ2 test revealed no statistically significant differences in total mortality (P = .07) or myocardial infarction (P = .13). The trial was not published in peer-reviewed literature, and details of randomisation processes, blinding, and handling of dropouts could not be obtained despite attempts to contact the original author."

Assessor's comments
To date, no relevant data allow to recommend garlic use for secondary prevention or/and clinical improvement neither in patients with coronary heart disease nor peripheral arterial occlusive disease.

Assessor's conclusion

There is no evidence of clinical benefit to support a well established use of garlic use in cardiovascular prevention. Data are insufficient to grant any well-established use indication.

Treatment of upper respiratory infections

No clinical data to support this indication is available.

d. Prevention and treatment of symptoms of common cold

Allium sativum being traditionally used for alleviation of symptoms of common cold in adults and children over 12 years, a research on this effect has been made. Indeed, in Sweden, garlic is considered as a traditional herbal medicinal product used for the relief of cold symptoms since 1985. Moreover, the British Herbal Pharmacopoeia considers that garlic is indicated for recurrent colds and whooping cough since 1983.

The published literature on this activity is sparse. A recent Cochrane meta-analysis (Lissiman and al., 2012) tried to determine whether garlic is effective for either prevention or treatment of the common cold, when compared to placebo, no treatment or other treatments. Authors of the meta-analysis conclude that there is insufficient clinical trial evidence regarding the effects of garlic in preventing or treating the common cold. A single trial suggested that garlic may prevent occurrences of the common cold but more studies are needed to validate this finding.

Standardised preparations

Clinical trials of interest

Josling 2001: Of the six trials identified as potentially relevant in the above Cochrane meta-analysis, only one trial met the inclusion criteria (randomised controlled trial): Josling, 2001. This trial randomly assigned 146 participants to either a garlic supplement (with 180 mg of allicin content) or a placebo (once daily) for 12 weeks. The trial reported 24 occurrences of the common cold in the garlic

intervention group compared with 65 in the placebo group (P < 0.001), resulting in fewer days of illness in the garlic group compared with the placebo group (111 versus 366). The number of days to recovery from an occurrence of the common cold was similar in both groups (4.63 versus 5.63).

Assessor's comments

The posology in the study is one garlic capsule, containing 180 mg of powder per day (name of the speciality: Allimax) with the main meal.

The trial of Josling, 2001 has good methodological aspects:

- *It is a randomized double-blind placebo study*

- *Participants were matched for age, sex and previous use of garlic. Demographic characteristics of patients are well-balanced between the two groups.*

- *The research co-ordinator was blinded for the duration of the trial*

Nevertheless the statistical analysis and primary endpoint seem to be not predefined in advance. The patients recorded general well-being for 3 months by using a five-point scale (5 = well, no problems; 4 = quite well with occasional sneeze, not disruptive to normal routine; 3 = can feel a cold coming on, some minor symptoms; 2 = feeling low and beginning to exhibit symptoms; 1 = full cold symptoms [headache, sneezing, runny nose, tiredness). This kind of scale is subjective as it was not confirmed by any objective observation.

Nantz (2012): More recently, a randomised, double-blind, placebo-controlled trial was performed with the aim to demonstrate that aged garlic extract could modify proliferation of γδ-T cell function and NK cells that are known to modify immunity in humans after 45 days of treatment. A secondary outcome assesses the incidence of colds and flu after 90 days of treatment.

This trial randomly assigned 120 participants to either a garlic supplement (with 2.56 g/day of aged garlic extract) or a placebo (once daily).

After 45 days of consuming an encapsulated aged garlic extract, γδ-T cells (p = 0.039) and NK cells (p = 0.043) were shown to proliferate better compared to placebo. After 90 days of supplementation, illness diary entries showed that the incidence of colds and flu were not statistically different. However, the group consuming the aged garlic extract appeared to have reduced severity as noted by a reduction of 21% in the number of symptoms reported and a reduction of 61% in the number of days where the subjects functioned sub-optimally:

Table 14: Characteristics of illnesses (cold and flu symptoms) over 90 days (Nantz *et al.*, 2012)

	Placebo (n = 56)	Age (n = 56)	p value
Number of people that got ill/not ill	28/28	26/30	0.848
Reported incidence of illnesses (percent)	58 (55%)	48 (45%)	0.442
Number of days having symptoms in the group that got sick	358	317	0.132
Total number of symptoms reported during the study	737	584	<0.001
Average number of symptoms per illness incident	14.0 ± 17.6[b]	11.9 ± 17.0	0.536
Reported incidence of DIA[c]	38	15	<0.001
Total days of DIA during the study	126	53	<0.001
Number of work days missed	19	8	0.035
Total number of visits to doctor	4	8	0.221

Assessor's comments

The results suggest that garlic could reduce the severity of cold and flu symptoms but not the incidence of these events. The reduction of severity of cold and flu symptoms is not a predefine outcome so this observation is only exploratory. Moreover, the assessment of illness is based on a self-reporting patient without any confirmation of pathogen presence for example.

Assessor's conclusion

There is a need for large, high-quality randomised controlled trials to support a general indication for common cold. Nevertheless, a traditional use for prevention or treatment of symptoms of common cold could be justified as the product has a traditional use since 1985 in Sweden and 1987 in UK.

e. Antiglycaemic effects

The exact mechanism of garlic as antidiabetic agent is still not clear. It is suggested that garlic can act by increasing either the pancreatic secretion of insulin from the beta cells or its release from bound insulin (Jain *et al.*, 1975).

Standardised and non standardised preparations (mixed data)

Hypoglycaemic effect of garlic in human is not well studied. In healthy patients, four interesting studies versus placebo have been published:

Table 15: Hypoglycaemic effect of garlic versus placebo in four trials in healthy patients

References	Preparation	Dose	Design	Duration	Results on glucose levels
Zhang *et al.*, 2001	Garlic oil & garlic powder	8.2 mg/day and 7.8 mg/day	Double blind, versus placebo	11 weeks	-0.16 mmol/L -0.04 mmol/L
Bordia *et al.*, 1998	Ethyl acetate extract from 1 g peeled and crushed raw garlic		Versus placebo	3 months	No difference on FBG
Jain *et al.*, 1993	Garlic powder	900 mg/day	Double blind, Versus placebo	12 weeks	No difference on FBG
Kiesewetter *et al.*, 1991	Garlic powder	800 mg/day	Double blind, versus placebo	4 weeks	Versus baseline: -10.4 mg/dl

Assessor's comments

Studies reporting hypoglycaemic effects of garlic in healthy subjects are scarce and conflicting. The references suffer from critical methodological limitations (low number of patients, no statistical analyse provided, incomplete results) precluding any formal conclusion on this property.

In diabetic's patients, 2 old randomised studies versus placebo have been published:

Table 16: Hypoglycaemic effect of garlic versus placebo in two trials in diabetic's patients

References	Design	Sample	Intervention	Control	Duration	Outcomes
Sitprija S *et al.*, 1987	Double-blind: 2 parallel groups	33 Type 2 diabetic's patients	700 mg/d (preparation unspecified)	Placebo	4 weeks	No change in Fasting Blood Glucose or Post-Prandial glucose or insulin
Mansell P. *et al.*, 1996	Double blind: 2 parallel groups	60 Type 2 diabetic's patients	3x300 mg of dried garlic tablets	Placebo	12 weeks	No significant change in FBG, HbA1c, serum insulin or C peptide.

Assessor's comments

It is difficult to appreciate the real quality of these old studies through the associated publications but the results suggest that garlic has no antidiabetic's properties.

Sobenin (2008): hypoglycaemic potential of garlic in patients with type 2 diabetes was reported in a 4-week double-blinded placebo controlled study in 60 patients. Theses results contradict with those obtained by Atkhami and al (2006) who reported that garlic has no hypoglycaemic effect in 45 type 2 diabetic's patients treated with 300 mg three times a day for 4 weeks.

Standardised preparations

Asharf (2011): More recently, two new studies have been published by the same team (Asharf *et al.*).

* Effects of garlic on blood glucose levels and HbA1c in patients with type 2 diabetes mellitus. Rizwan Ashraf M. Phil, Rafeeq Alam Khan and Imran Ashraf. Journal of medicinal Plants Research. July, 2011:

Assessor's comment

Considering that this study is the first comparing garlic with adequate comparators (placebo and metformin) and that the duration of the study is the longest available in patients with type 2 diabetes mellitus, the Rapporteur's will particularly describe this study.

Aim

The aim of the study was to evaluate the effects of garlic on fasting blood glucose and HbA1c in patients with type 2 diabetes.

Study design

This was a 24 week, single-blind placebo controlled study. Patients with previously diagnosed type 2 diabetes mellitus (n=210) were enrolled and divided randomly into 7 groups: A, B, C, D, E, F and G, each comprised 30 patients. Patients in group A, B, C, D and E were given garlic tablets at the dose of 300, 600, 900, 1200 and 1500 mg per day. Group F was given tablet metformin 500 mg twice daily while patients in group G were given placebo.

Assessor's comments

The choice of the comparators (metformin and placebo) is pertinent.

No statistical method has been made in order to calculate the sample sizes. No endpoints are specified. Randomisation and blinding procedures are not described. The trial is a single-blind trial.

Inclusion criteria

Patients with diagnosed type 2 diabetes mellitus of either sex with fasting blood sugar levels above 126 mg/dl. Patients aged between 25 to 70 years.

Exclusion criteria

The patients were excluded from the study if they are, known to have history of allergy to garlic, pregnant or lactating women, patients with type 1 diabetes mellitus, patients having history of myocardial infarction, coronary artery bypass grafting, established coronary artery disease, unstable angina, clinically manifest heart failure, patients with history of liver disease and impaired renal function and if they are known to have any other concurrent medical illness.

Results

Disposition of patients

A total of 210 patients were initially enrolled in the study. 195 patients were associated throughout the study and completed the protocol till week 24. Out of the dropped 15 patients, 3 patients were dropped from metformin treated group, 2 patients due to vague abdominal discomforts and refused to continue the protocol while 1 patient complained of weight loss and did not come for follow up after 12 weeks. The remaining patients were dropped from different garlic treated groups, 8 patients did not come for follow-up due to unknown reasons, 4 patients refused to give the blood samples for biochemical analysis so were forbidden to continue the study. 3 patients were dropped in the garlic treated group with 1500 mg, who reported heart burn in the first week of study and refused to take any further treatment.

Assessor's comments
Discontinuation rates were rather low.

Baseline demographics and background characteristics

Table 17: Baseline characteristics of patients (Ashraf, 2011)

	Placebo treated group	Garlic treated group	Metformin treated group
Men	15	90	17
Women	15	60	13
Age (years)	45 ± 4.58	40 ± 5.04	50 ± 5.80
Body weight (Kg)	69.1 ± 7.58	68.2 ± 10.45	65.4 ± 9.80
Height (cm)	166.4 ± 6.58	165.2 ± 8.81	167.60 ± 9.20
Average duration of type 2 diabetes mellitus	3 years	3 years	2.5 years
Co-morbid disease	None	None	None

Assessor's comments
Patients were clearly older in metformin treated group (50 years old) and in placebo group (45 years old) than in garlic treated group (40 years old). All the patients in the placebo group are women. Therefore the method of randomization is questionable.

The trial did not explicitly exclude patients on antidiabetic's medication at the inclusion.

Effects on fasting blood glucose and HbA1c

Table 18: Effects of garlic on FPG (Ashraf, 2011)

Group according to dose	Fasting blood sugar (mg/dl)		
	Week 0	Week 12	Week 24
(Group A) Garlic 300 mg	127 ± 0.334 (n = 30)	126 ± 0.360** (n = 27)	125 ± 0.379** (n = 27)
(Group B) Garlic 600 mg	128 ± 0.311 (n = 30)	127 ± 0.369** (n = 27)	126 ± 0.446** (n = 27)
(Group C) Garlic 900 mg	128 ± 0.263 (n = 30)	126 ± 0.274** (n = 27)	124 ± 0.289** (n = 27)
(Group D) Garlic 1200 mg	128 ± 0.315 (n = 30)	125 ± 0.264** (n = 27)	123 ± 0.263** (n = 27)
(Group E) Garlic 1500 mg	129 ± 0.223 (n = 30)	126 ± 0.213** (n = 27)	123 ± 0.225** (n = 27)
(Group F) Metformin 500 mg	125 ± 1.246 (n = 30)	119 ± 1.243** (n = 27)	118 ± 1.045** (n = 27)
(Group G) Placebo	127 ± 0.192 (n = 30)	129 ± 0.241** (n = 27)	131 ± 0.363** (n = 27)

Data are presented as mean ± standard error. Asterisk denotes significant p values. * Significant $p < 0.05$ ** Highly significant $p < 0.005$.

Table 19: Effects of garlic on HbA1c (Ashraf, 2011)

Group	Blood levels of HbA1c (%)			
	Week 0	Week 12	Week 24	Mean difference
Group A (300 mg dose of garlic)	6.59 ± 0.044	6.26 ± 0.044**	6.02 ± 0.048**	- 0.57
Group B (600 mg dose of garlic)	6.52 ± 0.055	6.33 ± 0.064	6.06 ± 0.054**	- 0.46
Group C (900 mg dose of garlic)	6.60 ± 0.055	6.31 ± 0.053*	6.03 ± 0.054**	- 0.57
Group D (1200 mg dose of garlic)	6.53 ± 0.056	6.26 ± 0.045	6 ± 0.053**	- 0.53
Group E (1500 mg dose of garlic)	6. 73 ± 0.035	6.32 ± 0.041**	5.97 ± 0.032**	- 0.76
Group F (Metformin 500 mg)	6.64 ± 0.044	6.26 ± 0.036**	6.21 ± 0.042**	- 0.43
Placebo	6.31 ± 0.036	6.37 ± 0.035	6.40 ± 0.049	+ 0.09

* Significant $p < 0.05$, ** Highly significant $p < 0.005$, (-) indicates decrease in mean HbA1C from week 0 to week 24. (+) indicates increase in mean HbA1C from week 0 to week 24.

Assessor's comments

Patients should be rather considered as pre-diabetic's patients regarding baseline FBG and HbA1c. In the metformin group, the mean FBG is 125 mg/dl whereas the inclusion criterion was "fasting blood sugar levels above 126 mg/dL".

The number of patients analysed is unknown. It is unclear if an intention-to-treat analysis has been used.

A low difference is observed in FBG between baseline and week 24 (maximum 6 mg/dL) in all the groups. Curiously, the difference in HbA1c between baseline and week 24 are more significant, about - 0.5% of difference in HbA1c in all the groups. The validity of the assay regarding HbA1c measurement is questionable.
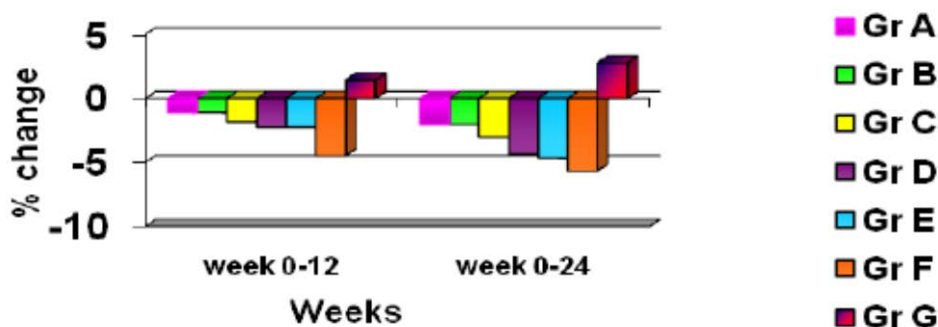

Figure 2. Percentage changes in FBS from week 0 to week 12 and from week 0 to week 24. Gr A = Garlic 300 mg, Gr B = Garlic 600 mg, Gr C = Garlic 900 mg, Gr D = Garlic 1200 mg, Gr E = Garlic 1500 mg, Gr F = Metformin, Gr G = Placebo. The (-) sign denote decrease in percentage.

Figure 6: Percentage changes in FBS (Ashraf, 2011)

Assessor's comments
Although the results indicate that garlic could be effective in lowering blood glucose, the low quality of this study on methodology aspects catches troubles on the validity of the results. This study could be considered at the best as an exploratory study.

*Garlic (Allium sativum) supplantation with standard antidiabetic agent provides better diabetic control in type 2 diabetes patients. Rizwan Asharfi, Rafeeq Alam Khan and Imran Ashraf. Pak. J. Pharm. Sci. October 2011:

The study was conducted in diagnosed type 2 diabetic patients (n=60) with fasting blood sugar level above 126 mg/dl to evaluate the effects of adding garlic tablets with standard antidiabetic therapy on blood sugar. Patients were divided randomly into 2 groups. Group 1 (n=30) was given tablet Garlic 300 mg thrice daily + Metformin 500 mg twice daily and Group 2 (n=30) was given Placebo+Metformin 500 mg twice daily respectively for 24 weeks. Fasting blood glucose were measured at week 0, 12 and week 24.

Group1 showed significant reduction in fasting blood sugar at week 24 with a percentage decrease of (-3.12%, from 128.3 mg/dl at baseline to 124.8 mg/dl) as compared to group 2 (-0.59%, from 112.9 mg/dl to 110.2 mg/dl).

Assessor's comments
The same methodological problems than the previous study are observed (randomisation, validity of the results).

Conclusion on the garlic effect on blood glucose reduction:

The data from the studies are contradictory and insufficient to conclude on a hypoglycaemic effect of garlic in healthy patients or in diabetic's patients.

f. Treatment of tick bites

A randomised, double-blind intervention trial of garlic (1200 mg per day of garlic in capsules) to prevent tick bites has been published (Stjernberg, 2000). Fifty individuals consumed garlic and 50 individuals consumed placebo for 8 weeks, followed by a wash-out period of 2 weeks, and then cross-over to placebo or garlic for another 10 weeks. In the intention-to-threat analysis, 66 (66%) of 100 participants recorded tick bites versus 55 (69%) of 80 participants in the per-protocol analysis. There was significant reduction in tick bites when consuming garlic compared with placebo in per protocol analysis (Wilcoxon test, $p=0.04$). A greater number of the participants were bitten by ticks during placebo consumption (normal approximation of binomal assumption, relative risk by intention to treat, 0.79 [95% confidence interval, 0.65-0.96]; relative risk per protocol, 0.70 [95% CI, 0.54-0.90]).

The authors suggested that garlic may be considered as a tick repellent for individuals and populations at high risk for tick bite.

Assessor's comments
There is no information about the garlic composition (fresh, dried or treated garlic). There is no description of the population studied, no information of the method of randomisation. The statistical analysis is lacking, which is problematic in a cross-over study.

Conclusion on the prevention of tick bites

Data are insufficient to grant an indication for prevention of tick bites.

g. Cancer prevention

Only dietary garlic consumption was investigated in cancer prevention and both randomised studies a case control studies were published. Of them:

- Using data from an integrated network of Italian and Swiss case-control studies, Galeone C and al (Galeone C, 2006) analysed the relation between frequency of onion and garlic use and cancer at several sites. They calculated odds ratios (ORs) by using multivariate logistic regression models that were adjusted for energy intake and other major covariates.

  They reported that several epidemiologic investigations have suggested an inverse relation between intake of allium vegetables and stomach, colorectal, and prostate cancers (Ernst E 2000, Izzo AA, 2004), but they pointed the fact that results for other cancer sites are scanty, especially for Western countries: most studies were conducted in China, and the results of those studies are not easily applicable to Western populations, whose dietary habits are largely different; in particular, garlic intake is far lower (Hsing AW, 2002 ; Hu J, 1994 ; Fleischauer AT, 2000 ; Chan JM, 2005).

  Authors remind that the one of the early epidemiologic studies considering this issue was a case-control study of diet and gastric cancer conducted in Italy (Buiatti E, 1989) found an odds ratio (OR) for the highest consumption of garlic of 0.40; latter, 3 Chinese case-control studies confirmed the favorable role of allium vegetables (You WC, 1989 ; Gao CM, 1999 ; Setiawan VW, 2005); and the relative risk (RR) of stomach cancer for garlic consumption estimated by a meta-analysis (Fleischauer AT, 2000) was 0.54 (95% CI: 0.44, 0.66). That study also found a protective role of garlic against colorectal cancer, with a RR of 0.67 (95% CI: 0.56, 0.80), on the basis of 2 cohort and 4 case-control studies (Fleischauer AT, 2000). Allium vegetables were also inversely related to breast cancer in 2 European investigations. They also report that in a French case-control study of breast cancer (Challier B, 1998), the OR was 0.30 (95% CI: 0.17, 0.52) for the highest intake of allium vegetables, and a Swiss study found similar results (Levi F, 1993). Additionally, the observance that the role of allium vegetables in the etiology of prostate cancer has been rarely studied. One study found an OR for the highest intake versus non use of 0.64 (95% CI: 0.38, 1.09) (Key TJ, 1997), while in a recent Chinese study, the OR was 0.51 (95% CI: 0.34, 0.76) (Hsing AW, 2004). The studies that examined the issue on upper aerodigestive tract cancers were conducted in China and found a protective role of allium vegetables, with ORs varying between 0.3 and 1.1 (Hu J., 1994; Gao CM, 1999; Gao YT, 1994, Zheng W, 1992). Furthermore, a recent US study reported an inverse relation between allium vegetable intake and the risk of pancreatic cancer (OR = 0.46; 95% CI: 0.33, 0.63) (Chan JM, 2005). To provide further information on the issue, we have analysed the relation between the frequency of onion and garlic use and the risk of cancer at various sites, using data from an integrated network of case-control studies conducted in southern Europe.

  Subjects and methods

  Between 1991 and 2004, they conducted a series of case-control studies on several neoplasms in various areas of northern, central and southern Italy. Studies on cancers of the oral cavity and pharynx, esophagus, large bowel, larynx, and breast were also conducted in the Swiss Canton of Vaud. Data were obtained with the same design, questionnaire, and inclusion criteria (Levi F, 1993; Franceschi S, 1999; Levi F, 2000; Bosetti C, 2000; Franceschi S, 1997; Bosetti C, 2002; Franceschi S, 1995; Bosetti C, 2001; Bosetti C, 2004). All studies included only incident, histologically confirmed cases.

The first study, on cancer of the oral cavity and pharynx, included 749 cases (median age: 57 y) and 1772 controls (median age: 57 y). The second study, on cancer of the esophagus, included 395 cases (median age: 60 y) and 1066 controls (median age: 60 y). The third study, on the large bowel, included 1394 cases of colon cancer (median age: 62 y), 886 cases of rectal cancer (median age: 63 y), and 4765 controls (median age: 58 y). The fourth study, on laryngeal cancer, included 527 cases (median age: 61 y) and 1297 controls (median age: 61 y). The fifth study, on breast cancer, included 2900 cases (median age: 55 y) and 3122 controls (median age: 56 y). The sixth study, on cancer of the ovary, included 1031 cases (median age: 56 y) and 2411 controls (median age: 57 y). The seventh study, on prostate cancer, included 1294 cases (median age: 66 y) and 1451 controls (median age: 63 y). The eighth study, on renal cell cancer, included 767 cases (median age: 62 y) and 1534 controls (median age: 62 y).

Controls were admitted to the same network of hospitals as cases for a wide spectrum of acute, nonneoplastic conditions unrelated to known or potential risk factors for the corresponding cancer site considered or to long-term diet modification (overall, 24% had traumas, 29% had nontraumatic orthopedic disorders, 21% had acute surgical conditions, and 26% had miscellaneous other illnesses). All cases and controls were <80 y old and were identified and questioned by trained interviewers during their hospital stay in a network of teaching and general hospitals in the areas under surveillance. The proportion of refusals was <5% among cases and controls in all Italian centers and ≈15% in Switzerland.

Interviewers used a structured questionnaire that included information on socio-demographic factors, anthropometric variables, smoking, alcohol and other lifestyle habits, a problem-oriented medical history, physical activity, and history of cancer in relatives. Information on diet referred to the 2 y preceding diagnosis and was based on a food-frequency questionnaire (FFQ) that was validated for nutrient intake and tested for reproducibility for specific nutrients and food items. The FFQ included 78 foods, food groups, or recipes and allowed the estimation of energy intake (Franceschi S, 1993; Decarli A, 1996). The average weekly frequency of consumption of foods was elicited, and therefore the FFQ looked more like a menu from a restaurant than a shopping list. Frequencies of less than once per week but at least once per month were coded as 0.5 per week, whereas never or less than once per month was coded as 0. The FFQ was divided into 6 sections: 1) bread, cereals, and first courses; 2) second courses (ie, meat, fish, and other main dishes); 3) side dishes (ie, vegetables and fried or baked potatoes); 4) fruit; 5) sweets, desserts, and soft drinks; and 6) milk, hot beverages, and sweeteners. At the end of each section, 1 or 2 open questions were used to include foods that were not in the questionnaire but were eaten at least once per week.

Among the items in the FFQ, 2 questions referred specifically to consumption of onion and garlic. For frequency of onion use, we asked for the weekly frequency of consumption and usual portion size (small, medium, large), for which an intermediate portion corresponded to 80 g onion. For frequency of garlic use, we asked for the common consumption as a qualitative variable, scored as 1 for nonuse or low use, 2 for intermediate use, and 3 for high use. No additional information on the type of garlic and onion and on manner of using (fresh, powders, or garlic supplements) was available.

Statistical analyses

ORs and the corresponding 95% CIs of selected cancers according to the different frequencies of onion use (never, ≤1, >1 to <7, and ≥7 portions/wk) and garlic use (nonuse-low, moderate, and high) were derived by using unconditional multiple logistic regression (in unmatched studies, ie, breast, ovarian, colorectal, and prostate cancers) or conditional multiple logistic regression (in studies matched for age, sex, and study center, ie, upper aerodigestive tract and renal cell

cancers) (Breslow NE , 1980). All regression models included terms for age, sex (where appropriate), study center, education, body mass index, and energy intake. According to the cancer site analysed, further adjustments were made for alcohol drinking, smoking habit, physical activity, parity, and family history of cancer at the same site.

Results

The distribution of cases and controls according to sex, age, and information on selected dietary aspects in the studies analysed are shown in Table 1. For each cancer study, cases consumed both vegetables and onions less frequently than did controls, except for vegetable intake in the prostate cancer study. Consumption of onions varied between 0–14 and 0–22 portions/wk among cases and controls, respectively. Garlic use was lower in cases in all studies, except for the breast, ovary, and prostate cancer studies, for which mean values were not significantly different.

Table 20: Sex and age distribution and information on selected dietary aspects in the studies investigated garlic and onion (Galeone, 2006)

TABLE 1
Sex and age distribution and information on selected dietary aspects in the studies investigated, Italy and Switzerland, 1991–2004

Cancer site	Calendar period	Male <60 y	Male ≥60 y	Female <60 y	Female ≥60 y	Energy intake	Total vegetable intake	Onion intake	Garlic use
		n		n		kcal/d	portions/wk	portions/wk	intake score
Oral cavity and larynx	1991–1997								
Cases		373	261	66	49	2326 ± 732[1]	8.6 ± 5.1	0.8 ± 1.3 (0–9)[2]	1.5 ± 0.7
Controls		698	554	323	197	2359 ± 780	12.0 ± 6.3	0.9 ± 1.8 (0–18)	1.7 ± 0.7
P[3]						0.29	<0.01		
Esophagus	1992–1999								
Cases		165	186	22	22	2333 ± 878	8.2 ± 5.3	0.9 ± 1.2 (0–7)	1.6 ± 0.7
Controls		429	446	84	107	2314 ± 767	11.5 ± 6.3	1.3 ± 2.0 (0–18)	1.7 ± 0.7
P						0.69	<0.01		
Large bowel	1992–2001								
Cases		477	841	404	558	2280 ± 753	10.5 ± 5.8	0.8 ± 1.3 (0–14)	1.6 ± 0.7
Controls		1259	1144	1379	983	2211 ± 744	11.5 ± 6.5	1.0 ± 1.6 (0–18)	1.7 ± 0.7
P						<0.01	<0.01		
Larynx	1992–2000								
Cases		205	273	25	29	2507 ± 757	9.4 ± 5.4	0.7 ± 1.1 (0–9)	1.6 ± 0.7
Controls		455	597	125	120	2334 ± 804	11.8 ± 7.2	1.0 ± 1.6 (0–18)	1.7 ± 0.7
P						<0.01	<0.01		
Breast	1991–2001								
Cases		—	—	1873	1063	2196 ± 645	12.2 ± 5.8	0.9 ± 1.5 (0–14)	1.7 ± 0.7
Controls		—	—	1862	1260	2098 ± 684	12.8 ± 7.0	1.0 ± 1.5 (0–22)	1.7 ± 0.8
P						<0.01	<0.01		
Ovary	1992–1999								
Cases		—	—	638	393	2233 ± 616	11.9 ± 6.8	0.5 ± 0.8 (0–9)	1.8 ± 0.7
Controls		—	—	1414	997	2104 ± 691	12.3 ± 7.3	0.6 ± 1.2 (0–22)	1.8 ± 0.8
P						<0.01	<0.01		
Prostate	1991–2002								
Cases		219	1075	—	—	2639 ± 746	10.9 ± 7.0	0.7 ± 1.0 (0–9)	1.7 ± 0.7
Controls		431	1020	—	—	2555 ± 761	10.3 ± 5.6	0.8 ± 1.2 (0–12)	1.7 ± 0.7
P						<0.01	0.02		
Renal cell cancer	1992–2004								
Cases		220	274	103	170	2408 ± 743	9.9 ± 5.6	0.6 ± 1.0 (0–7)	1.6 ± 0.7
Controls		440	548	213	333	2404 ± 779	10.9 ± 5.8	0.7 ± 1.2 (0–12)	1.8 ± 0.7
P						0.75	<0.01		

[1] $\bar{x}$ ± SD (all such values).
[2] Range in parentheses (all such values).
[3] Calculated by using a Student's t test for energy intake and a Wilcoxon's rank-sum test for total vegetable intake.

Shown in Table 18 for each cancer site considered are the distribution of cases and controls and the ORs and 95% CIs for subsequent frequencies or score of use of onion and garlic. Significant inverse associations were obtained for several cancer sites: the ORs for the highest versus the lowest category of use of onion were 0.44 (95% CI: 0.28, 0.67) for cancer of the large bowel, 0.17 (95% CI: 0.05, 0.61) for cancer of the larynx, and 0.27 (95% CI: 0.08, 0.85) for cancer of the ovary. For the frequency of use of garlic, the ORs for the highest versus the lowest category were 0.61 (95% CI: 0.44, 0.85) for cancer of the oral cavity or pharynx, 0.43

(95% CI: 0.28, 0.67) for cancer of the esophagus, 0.74 (95% CI: 0.63, 0.86) for cancer of the large bowel, 0.56 (95% CI: 0.38, 0.82) for cancer of the larynx, 0.81 (95% CI: 0.64, 1.00) for cancer of the prostate, and 0.69 (95% CI: 0.53, 0.92) for renal cell cancer. These results did not substantially change when they adjusted the multivariate models for total vegetable intake. None of the ORs was above unity. No significant direct relations were observed.

Table 21: Distribution of cases and controls and OR (Galeone, 2006)

TABLE 2

Distribution of cases and controls and odds ratios (ORs) and 95% CIs of selected cancers according to frequency of onion use (portions/wk) and garlic use, Italy and Switzerland, 1991–2004[1]

Cancer site	Onion use					Garlic score			
	Nonusers[2]	≤1	>1 to <7	≥7	P for trend	None or low[2]	Intermediate	High	P for trend
Oral cavity and pharynx[3]									
Cases:controls	346:890	270:527	128:302	5:53	—	403:789	266:701	79:280	—
OR (95% CI)	1	1.04 (0.81, 1.33)	0.78 (0.54, 1.11)	0.16 (0.06, 0.46)	0.02	1	0.81 (0.64, 1.02)	0.61 (0.44, 0.85)	0.002
Esophagus[3]									
Cases:controls	101:334	203:407	89:281	2:43	—	211:469	143:407	41:188	—
OR (95% CI)	1	1.46 (1.04, 2.06)	1.30 (0.85, 2.01)	0.12 (0.02, 0.58)	0.87	1	0.88 (0.65, 1.21)	0.43 (0.28, 0.67)	<0.001
Large bowel[4]									
Cases:controls	1,037:2,020	795:1,612	415:1,022	33:111	—	1,057:2,048	889:1,876	334:837	—
OR (95% CI)	1	0.86 (0.76, 0.97)	0.62 (0.53, 0.73)	0.44 (0.28, 0.67)	<0.0001	1	0.88 (0.78, 0.98)	0.74 (0.63, 0.86)	<0.0001
Larynx[3]									
Cases:controls	224:471	218:491	81:314	4:21	—	262:587	204:478	60:230	—
OR (95% CI)	1	0.79 (0.60, 1.05)	0.44 (0.30, 0.63)	0.17 (0.05, 0.61)	<0.0001	1	0.87 (0.67, 1.14)	0.56 (0.38, 0.82)	0.005
Breast[5]									
Cases:controls	1,338:1,360	949:1,008	563:683	50:71	—	1,297:1,450	1,093:1,109	510:561	—
OR (95% CI)	1	0.95 (0.84, 1.07)	0.89 (0.76, 1.04)	0.75 (0.50, 1.12)	0.08	1	1.07 (0.95, 1.20)	0.90 (0.77, 1.05)	0.43
Ovary[5]									
Cases:controls	555:1,261	368:822	104:310	4:18	—	414:1,035	439:911	178:462	—
OR (95% CI)	1	1.04 (0.87, 1.25)	0.57 (0.43, 0.75)	0.27 (0.08, 0.85)	0.0005	1	1.08 (0.90, 1.30)	0.78 (0.62, 0.98)	0.10
Prostate[6]									
Cases:controls	576:608	483:576	232:254	3:12	—	635:661	446:526	213:263	—
OR (95% CI)	1	0.89 (0.75, 1.06)	0.92 (0.72, 1.16)	0.29 (0.07, 1.03)	0.05	1	0.91 (0.76, 1.08)	0.81 (0.64, 1.00)	0.05
Renal cell cancer[7]									
Cases:controls	391:736	269:534	103:254	4:10	—	367:637	297:637	103:260	—
OR (95% CI)	1	0.92 (0.75, 1.13)	0.75 (0.56, 1.01)	0.62 (0.18, 2.10)	0.05	1	0.79 (0.65, 0.96)	0.69 (0.53, 0.92)	0.003

[1] The sums may not add up to the total number of cases and controls of each study because of a few missing values.
[2] Reference category.
[3] Estimates from conditional multiple logistic regression conditioned on age, sex, and study center and adjusted for education, BMI, energy intake, alcohol consumption, and smoking habit.
[4] Estimates from unconditional multiple logistic regression adjusted for age, sex, study center, education, BMI, energy intake, alcohol consumption, smoking habit, and physical activity.
[5] Estimates from unconditional multiple logistic regression adjusted for age, study center, education, BMI, energy intake, family history of breast or ovarian cancer, and parity.
[6] Estimates from unconditional multiple logistic regression adjusted for age, study center, education, BMI, energy intake, and family history of prostate cancer.
[7] Estimates from conditional multiple logistic regression conditioned on age, sex, study center, and date of interview and adjusted for education, BMI, energy intake, and smoking habit.

The authors considered consumption of onions and garlic in strata of age and sex (data not shown). The estimates were not significantly different across strata of the considered covariates for each cancer site, except for onion consumption in strata of age in the laryngeal study (P = 0.01) and in strata of sex in the esophageal study (P = 0.04) and for garlic consumption in strata of age in the ovarian study (P = 0.01). The 3-factor interaction (sex, age, and frequency of onion or garlic use) was not significant for any cancer site, with the exception of larynx cancer (P = 0.005) and onion use.

Authors discussion

The authors stated that they found in their study a protective role of a moderate frequency of onion consumption against colorectal, laryngeal, and ovarian cancers. The inverse relation was even more evident for high frequency of use, when it was also significant for oral cavity and esophageal cancers, but not for prostate, breast, or renal cell cancers. A moderate frequency of garlic consumption was inversely related to colorectal and renal cell cancers, and a high frequency of garlic consumption was significantly and inversely related to all cancer sites, except again for breast and prostate. These latter cancers are mainly associated with hormonal and reproductive factors, whereas their relation with dietary factors is inconsistent and still open to discussion (Henderson BE, 2000).

Onion and garlic intakes could be simply considered markers of a healthier lifestyle, which may include complex aspects of quantity and quality of diet. In fact, several of the cancer sites considered have been inversely related to intake of vegetables in several studies (Levi F, 1993; Franceschi S: 1999; Bosetti C, 2000; Franceschi S; 1997; Levi F, 1999; Bosetti C; 2002; Franceschi S 1995; Bosetti C, 2001; Bosetti C, 2004, and the evidence is more convincing for cancers of the oral cavity and pharynx, esophagus, and large bowel (Yang CS, 2001). For this reason, the authors adjusted all the models for total vegetable intake, but the results did not substantially change. The authors adjusted for selected major lifestyle and nutritional factors in the analysis, including alcohol use, tobacco use, and physical activity and were unable to explain the association observed. However, it is still conceivable that a complex combination of favorable correlates of *Allium* vegetable intake may have contributed to the apparent protection at several cancer sites. In fact, in the Italian diet, onion and garlic are often eaten or cooked in combination with foods such as tomatoes and olive oil in salads and sauces for pasta, and some studies reported that the synergistic action of garlic and tomato could have a preventive effect against the carcinogenic process (Sengupta A, 2004 ; Bhuvaneswari V; 2005).

The authors stated that they tried to minimise selection bias by excluding all control patients with diagnoses linked to long-term changes in diet or admitted for chronic conditions. Furthermore, they pointed that findings of the study cannot be due to a selectively higher response rate of health-conscious control subjects (Vastag B, 2005), because participation was practically complete for both cases and controls. An important limitation of our study is that we collected no information on the variety of onions and type of garlic used and on modalities of cooking. Among the strengths of these studies are the large number of subjects included; the similar interview setting of cases and controls, which provides reassurance against potential information bias (D'Avanzo B, 1997); the ability to adjust for major selected potential confounding factors; and the use of a validated and reproducible FFQ (Franceschi S, 1993 ; Decarli A , 1996) In particular, the Spearman correlation coefficient for reproducibility of frequency of onion use was 0.48, and the concordance of the subjective question on garlic use was well reproducible in >70–80% of the subjects (Franceschi S, 1993). The Pearson correlation coefficient for the validity of total energy intake was 0.63 (Decarli A, 1996)

Because the role of allium vegetables on cancer risk has been rarely studied, especially in Western countries, the authors considers that their study is original and of particular interest. Our findings confirm the indications of mainly Chinese studies of a protective role of allium vegetables on the risk of several common cancers.

Assessor's comment

Although the results of this study are interesting, the specific role of garlic can not be distinguished as the authors considered the common use of both garlic and onion. Otherwise, this study aims to investigate impact of dietary habits regarding garlic/oinion consumption on cancer prevention and not impact of garlic/oinion supplementation on cancer prevention.

In addition, as pointed by the authors, onion and garlic intakes could be simply considered markers of a healthier lifestyle, which may include complex aspects of quantity and quality of diet. The other limitation of this study is that no information on the variety of onions and type of garlic used and on modalities of cooking were collected.

PIC-EURGAST case control study (González CA, 2006): In 521,457 subjects participating to the EPIC cohort in 10 European countries, information of diet and lifestyle was collected at baseline. After an average of 6.5 years of follow-up, a total of 330 gastric cancer and 65 adenocarcinoma of oesophagus was used for the analysis. The relation between fruit and vegetable intake and gastric cancer/ adenocarcinoma of oesophagus. No association with total vegetable intake or specific groups of vegetables and gastric cancer risk, except for the intestinal type, where a negative association is possible regarding total vegetable (calibrated HR 0.66; 95% CI 0.35-1.22 per 100 g increase) and onion and garlic intake (calibrated HR 0.70; 95% CI 0.38-1.29 per 10 g increase). No evidence of association between fresh fruit intake and gastric cancer risk was observed. Regarding adenocarcinoma of oesophagus, non significant negative association was found for vegetable intake).

Assessor's comment

No conclusion in favour of garlic consumption in gastric cancer prevention. The same potential bias regarding the fact that garlic intakes could be simply considered markers of a healthier apply also for this study

In the Shandong Intervention Trial (You WC 2006), 2 weeks of antibiotic treatment for Helicobacter pylori reduced the prevalence of precancerous gastric lesions, whereas 7.3 years of oral supplementation with garlic extract and oil (garlic treatment) or vitamin C, vitamin E, and selenium (vitamin treatment) did not In a 14.7-year follow-up for gastric cancer incidence and cause-specific mortality among the 3365 subjects randomised the Shandon Intervention Trial (Ma JL, 2012), garlic and vitamin treatments were associated with non-statistically significant reductions in gastric cancer incidence and mortality.

Assessor's comment

Garlic use was not associated to reduction gastric cancer incidence and mortality.

In a multicenter case-control study (Sam S, 2011), dietary intakes of red meat, fat, garlic, and tomato/tomato products, as well as thorough demographic and medical characteristics, were determined in 194 cases with the newly diagnosed prostate cancer and 317 controls, without any malignant disease, admitted to the same network of hospitals. For garlic consumption, a borderline reduction in risk was observed (OR: 0.58, 95% CI: 0.32-1.01; P = 0.05).

Assessor's comment

Interpretation limitations of this type of study and the marginal significance result does not allow any conclusion in favour of any cancer prevention effect of garlic consumption.

In a case control study investigating food intake and the occurrence of oesophageal squamous cell carcinoma (Chen YK, 2009), 343 patients with OSCC and 755 cancer-free control subjects were recruited. Results suggest that intake of raw onions/garlic have a significant inverse association with the risk of esophageal cancer.

Assessor's comment

Non conclusion on OSCC prevention could be drawn form the results of this study. As stated above, garlic intakes could be simply considered markers of a healthier lifestyle, which may include complex aspects of quantity and quality of diet. In addition, for raw onions/garlic, the authors reported hight rate of missing data from 42% to 63%.

Rapporteur conclusion

There is no evidence of clinical benefit to support either traditional or a well-established use of garlic use in cancer prevention.

4.3. Clinical studies in special populations (e.g. elderly and children)

Children

Cardiovascular effects

McCrindle and al., 1998 examined the effects of a commercial garlic extract on lipids in 30 children (8 to 18 y of age) who had first-degree relatives afflicted with familial hypercholesterolemia or premature atherosclerotic cardiovascular disease and a minimum fasting total cholesterol concentration higher than 185 mg/dL (4.8 mmol/L) in a double-blind, randomised, controlled trial (RCT). The extract was administered in 300-mg doses (containing 0.6 mg allicin) three times daily for 8 weeks. There were no significant improvements in total fasting cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, apolipoprotein B-100, homocysteine, or fibrinogen concentrations.

A small significant increase (10%) in serum apolipoprotein A-I was observed, although this was not a primary outcome of the study.

Upper respiratory tract infections

A randomised controlled trial comparing tablets of garlic to benzimidazole or placebo in children has been published (Andrianova, 2003). At the first stage, tolerance of garlic (600 mg/day) and its effects on acute respiratory diseases (ARD) morbidity were investigated in an opened 5-month study in 172 children aged 7-16 years compared to 468 controls. It was not observed that garlic induces gastrointestinal side effects in children while ARD morbidity was reduced 2-4-fold as compared to the controls.

At the second stage, the effects of garlic (300 mg/day) on ARD morbidity were investigated in a double-blind placebo-controlled randomised 5-month trial in 42 children aged 10-12 years in comparison with 41 placebo-treated children and 73 benzimidazole-treated children. Garlic reduced ARD morbidity 1.7-fold compared to placebo and 2.4-fold vs benzimidazole. There was no significant difference in ARD morbidity between placebo- and benzimidazole-treated groups.

Assessor's comments

The article is in Russian. The conclusions of the abstract are that garlic has no side effects. Data are insufficient to recommend precise dosages when treating children.

Assessor's conclusions on use of garlic in children

A paucity of good evidences supports the use of garlic in children. Additional and larger studies are needed to confirm the efficacy and safety of use of garlic in this specific population. Nevertheless, considering the adults clinical data, the fact that garlic is widely use for food and that garlic is approved in children in many countries (UK, Poland SE), garlic could be used in adolescents over 12 years.

4.4. Overall conclusions on clinical pharmacology and efficacy

There is no consensus regarding which constituents of garlic have major effects on particular cardiovascular risk factors *in vivo* and by what mechanism these effects are achieved. The relative component of raw garlic is affected by growth conditions such as soil composition.

The review of the clinical studies data reported in this assessment report show that they were inconclusive to a well established use any indication. Contradictory results could be linked to methodological shortcomings, the use of different formulations of garlic and different duration of the studies.

- **Antilipidemic effects**

The effect of garlic on cholesterol or other lipid parameters has been investigated in numerous trials and meta-analyses, with variable results. The overall quality of the studies performed before 2000 is poor, particularly due to the low number of patients included. The well-conducted study such as Gardner (2007) did not demonstrate a significant clinical effect of garlic on LDL-C or other plasma lipid concentrations in adults with moderate hypercholesterolemia.

In the meta-analysis from Khoo *et al.* in 2009, conclusions were that the available evidence from randomised controlled trial does not demonstrate any beneficial effects on serum cholesterol and it should be noted that population was clearly heterogeneous in term of disease severity (hypercholesterolaemic patients and healthy subjects). The separate analysis conducted for comparisons of garlic preparation in healthy and hypercholesterolaemic subjects show similar results.

In the meta-analysis of Ried in 2013, several garlic type was used in the clinical studies: the majority of trials use garlic powder (27 of 37 trials included) whereas aged garlic extract has been used in only 6 trials. The number of patients included is low. It should be particularly emphasised that no comparison with authorised medicinal drugs for the treatment of hypercholesterolemia has been performed. Subgroup analysis by single type of garlic preparation suggested a greater cholesterol-lowering effect for aged garlic extract than for garlic powder, and a borderline effect for garlic oil. This meta-analysis suggested that garlic could reduce total cholesterol to a modest extent (-15.25 mg/dL), reduce LDL cholesterol (-6.41 mg/dL) and increase HDL cholesterol (+1.49 mg/dL) and the subgroup analysis by type of products shown contradictory results. Nevertheless, all these results should be interpreted with caution as heterogeneity is high among all the meta-analysis performed, notably in subgroup analysis of trials of longer duration or with higher baseline cholesterol levels. There is no sensitivity analysis with an evaluation performed with or without studies that were identified as outliers.

Uptated meta-analsysis of Ried in 2016 did not provide new evidence regarding effects on serum cholesterol and the the same interpretation caution apply as for the previous Ried meta-analysis.

Even now with the results of IMPROVE-IT study, the positive correlation and causal relationship between serum low density lipoprotein cholesterol LDL-C and the risk of coronary heart disease (CHD) is still under discussion.

The diverse composition and amount of active sulfur compounds of different garlic preparations used in various trials could be responsible for the above mentioned inconsistent findings.

Inconsistent clinical evidence warrants more study before reaching convincing conclusions. Thus, data are insufficient to grant an indication for hypercholesterolemia, a well-performed study is needed.

- **Antihypertensive effects**

Hypertension is considered as major risk factor for several cardiovascular and related diseases as well as for diseases leading to a marked increase in cardiovascular risk.

The effects of garlic on blood pressure cannot be established. The meta-analysis and clinical trials performed by Ried *et al.* suggest that aged garlic extract could reduce blood pressure in individuals with hypertension. Nevertheless, the trials on the effects of garlic on blood pressure suffer of inadequate study designs, low number of patients included, short duration and methodological deficiencies. Thus, use of garlic cannot be recommended as antihypertensive advice for hypertensive patients in daily practice.

The most recent meta-analysis (Rohner *et al.,* 2015 and Xiong and *et al.*, 2015), even if they showed a statistically significant reduction in SBP and DBP in hypertensive individuals treated with garlic preparations, have several limitations which preclude to support an indication: high heterogeneity of the studies and the dosages/type of garlic preparation, several methods of blood pressure measures, small simple size.

The the updated meta-analysis

In conclusion, available data are insufficient to grant an indication for hypertension. A well-conducted clinical trial is needed.

However:

The trends observed in these studies suggest that garlic supplementation may produce mild benefits on:

The combination of these effects may support traditional use as an adjuvant for the prevention of atherosclerosis

- **Antithrombotic effects / Cardiovascular morbidity and mortality**

The *in vivo* anti platelet seems established, however the mixed data issued from randomised controlled clinical studies, quality weakness of some of them does not allow concluding on the anti-platelet effect of garlic. Moreover, even if an antiplatelet effect is considered, the clinical relevance of such effect should be assessed through morbi-mortality clinical trials (secondary prevention in patients with coronary heart disease, previous stroke, at high CV risk etc.)

The available data suggest a limited effect on increasing fibrinolytic activity, but no conclusion can be made. Therapeutic usefulness of such effect should be investigated through clinical trials with relevant clinical endpoints.

A slight effect on decreasing plasma viscosity is suggested by the clinical data, but no conclusion can be made. Therapeutic usefulness of such effect should be investigated through clinical trials with appropriate clinical endpoints.

These data support the hypothesis that garlic intake had a protective effect on the elastic properties of the aorta related to aging in humans. However, only clinical trials with strong clinical endpoints can demonstrate a clinical benefit.

There is no evidence of clinical benefit to support a well-established use of garlic use in cardiovascular prevention, the lake of clinical study with robust endpoint is the main issue.

- **Treatment of upper respiratory infections**

No clinical data to support this indication is available.

- **Prevention and treatment of symptoms of common cold**

Regarding the prevention or treatment of the common clold, even though the Cochrane meta-analysis (Lissiman and al., 2012) conclude that there is insufficient clinical evidence regarding the effects of garlic in preventing or treating the common cold, the sole study retained for the analysis (Josling, 2001) showed fewer days of illness in the garlic group compared with the placebo group. Moreover, another recent trial (Nantz, 2012) suggests that consuming the aged garlic extract could reduce severity of symptoms reported. Therefore, a traditional use for prevention or treatment of symptoms of common cold could be accepted.

- **Antiglycaemic effects**

The data from the studies are contradictory and insufficient to conclude on a hypoglycaemic effect of garlic in healthy patients or in diabetic's patients.

- **Treatment of tick bites**

Data are insufficient to grant an indication for prevention of tick bites.

- **Cancer prevention**

There is no evidence of clinical benefit to support either traditional or a well-established use of garlic use in cancer prevention.

In conclusion, the trends observed in the studies conducted for cardiovascular indication could suggest that garlic supplementation may produce mild benefits on: the levels of total cholesterol, triglycerides, and in a lesser extend to LDL-C, platelet aggregation, blood pressure and arteries stiffness prevention. However, based on the high morbi-mortality risk of cardiovascular disease (CVD), the combination of these effects are sufficient to support a traditional use as an adjuvant for the prevention of atherosclerosis.

Regarding the prevention or treatment of the common clold, even though the Cochrane meta-analysis (Lissiman and al., 2012) conclude that there is insufficient clinical evidence regarding the effects of garlic in preventing or treating the common cold, the sole study retained for the analysis (Josling, 2001) showed fewer days of illness in the garlic group compared with the placebo group. Moreover, another recent trial (Nantz, 2012) suggests that consuming the aged garlic extract could reduce severity of symptoms reported. Therefore, a traditional use for prevention or treatment of symptoms of common cold could be accepted.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

The safety of consuming small quantities of raw garlic is evident in its worldwide use as a culinary spice.

5.2. Patient exposure

According to the provided literature, no data are available

5.3. Adverse events, serious adverse events and deaths

According to ESCOP monograph, in rare cases gastro-intestinal irritation or allergic reactions occur. In published studies involving consumption of up to 1.2 g of garlic powder daily, garlic odour was the typical and most common side effect of garlic intake. The incidence might be as much as 50%. However, this effect is not considered to be adverse. Common side effects were gastrointestinal discomfort and in rare cases allergic reactions. In healthy volunteers, 10 g of raw garlic consumed daily for 2 months induced no adverse events. Daily administration of high doses of garlic oil (approx. 120 mg, equivalent to 60 g/day fresh garlic) over a period of 3 months did not result in any toxic effects or adverse events. In 3 cases reduced platelet aggregation and prolonged bleeding time have been reported after intake of raw garlic or garlic powder.

According to WHO monograph, Bulbus Allii Sativi has been reported to evoke occasional allergic reactions such as contact dermatitis and asthmatic attacks after inhalation of the powdered drug. Those sensitive to garlic may also have a reaction to onion or tulip. Ingestion of fresh garlic bulbs, extracts, or oil on an empty stomach may occasionally cause heartburn, nausea, vomiting, and diarrhoea. Garlic odour from breath and skin may be perceptible. One case of spontaneous spinal epidural haematoma, which was associated with excessive ingestion of fresh garlic cloves, has been reported (Rose *et al.*, 1990).

The adverse events reported in the reviewed clinical trials malodorous breath or body odor abdominal pain, fullness, anorexia, or flatulence. Otherwise, several case reports reported dermatitis, rhinitis, Meniere disease, asthma, myocardial infarction, bleeding, epidural hematoma, increased International Normalised Ratio in patient taking warfarin, small-intestine obstruction, esophageal and abdominal pain, and flatulence.

A survey by Koch (1995) showed that allergic reactions to garlic were reported in a total of 39 publications between 1938 and 1994 (Koch HP, 1995). Most of these cases involved an allergic contact dermatitis, sometimes severe (Eming SA, 1999), which has been reported in people with occupational exposure to garlic. There have also been sporadic reports of allergic conjunctivitis, rhinitis, or bronchospasms occurring in response to garlic inhalation or ingestion (Falleroni AE, 1981; Papageogiou D, 1983). Other reported side effects included bloating, headache, dizziness, and profuse sweating (Beck E; 1993).

Regarding bleeding risk, although the reviewed clinical data suggest that garlic could have antiplatelet activity, fibrinolytic activity and decreased blood viscosity, these data rule out that garlic is potent inhibitor of platelet aggregation. However, no relevant data are available regarding the potentiating effect of antiplatelet drugs (ASA, clopidogrel etc.) when co-administered with garlic. Additionally, spontaneous bleeding in were reported in several case reports:

- An 87-year-old man (not taking anticoagulants) developed a spontaneous epidural hematoma associated with significant garlic consumption (2 g, the equivalent of four cloves daily), with an elevated bleeding time upon admission that returned to normal 3 days after discontinuing the garlic (Rose, K. D, 1995)

- A 72-year-old man taking no medications other than garlic had bleeding after a transurethral prostate resection and required transfusion. Although not measured at the time of the bleeding incident, his platelet function was impaired when measured 3 months after resumption of the garlic tablets (German, K, 1995)

- A 32-year old woman taking no medications but with heavy garlic intake required evacuation of a hematoma after breast augmentation. She was noted to have a prolonged bleeding time

(12.5 minutes) before surgery, and 1 week after cessation of garlic her bleeding time returned to normal (6 minutes) (Burnham, B. E, 1995)

The reported cases of post operative bleeding and spinal hematoma (German K, 1995 ; Petry JJ, 1995 ; Burnham BE, 1995 ; Rose KD, 1990) have probably led to a trend in anaesthesia practice to suggest avoiding any garlic consumption 7 days before surgery, especially if postoperative bleeding is a particular concern (Tsen L , 2000 ; Kaye AD ; 2000 ; Ang-Lee MK, 2001 ; Ciocon JO, 2004 ; Messina BA ; 2006). However, 2 studies investigating inhibition of platelet aggregation (Scharbert G, 2007)

However, two clinical trials results (*Scharbert G, 2007; Beckert BW, 2007)* suggest that dietary garlic consumption does not affect platelet function.

Assessor's comment
In the absence of sufficient data, garlic consumption should be avoided 7 days before surgery, especially if high operative and postoperative bleeding risk.

Rapporteur conclusion: Garlic has been an integral part of our diet for long time; it is taken for granted that garlic is safe in a wide range of doses. However, data form clinicals trials and case reports highlight some of the adverse and toxic effects. Most of them are mild to moderate. In the absence of sufficient data bleeding risk should be cautiously considered in patients at high risk of bleeding.

Safety in children

According to a review of data conducted by the University of Alberta (Shamseer and al, 2006), burns or contact dermatitis are the most noted adverse effects of garlic used topically. Several studies have reported this adverse event in children. Patients from 3 months to 6 years of age reportedly have experienced second-degree burns after the topical application of raw, crushed garlic.

5.4. Laboratory findings

There are limited data regarding laboratory findings. As mentioned above and in the DDI paragraph, prolonged bleeding time, decrease of platelet aggregation and INR increase were reported.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

The use for children and adolescents under 18 years of age has not been established due to the lack of data for the use as an adjuvant for the prevention of atherosclerosis.

The use in children under 12 years of age has not been established due to the lack of data for the use for relief of the symptoms of cold.

5.5.2. Contraindications

Hypersensitivity to the active substance

Patients under saquinavir/ritonavir therapy (see also section 4.5 Interactions).

5.5.3. Special Warnings and precautions for use

Garlic consumption should be avoided 7 days before surgery because of the post-operative bleeding risk

5.5.4. Drug interactions and other forms of interaction

According to ESCOP monograph, in two cases an increased INR (International Normalised Ratio has been observed in patients on warfarin who had used garlic products (Sunter, 1991).

According to WHO monograph, patients on warfarin therapy should be warned that garlic supplements may increase bleeding times. Blood clotting times have been reported to double in patients taking warfarin and garlic supplements (Sunter, 1991).

According to the Natural Medicines Comprehensive Database monopraph, garlic should not be used with isoniazid (absorption of isoniazid can be reduce), with medications used for AIDS (Non-Nucleoside Reverse Transcriptase Inhibitors: neviparine, delavirdine, efavirenz) due to the fact that garlic can reduce their effectiveness. Moreover, garlic should be used with caution when it is associated with contraceptive drugs (garlic decrease the effectiveness of oral contraceptives), cyclosporine (some garlic preparations may interact with this drug but the information are lacking on this DDI), with CYP 2E1 subtracts (in fact garlic oil can increase the effects and the adverse effects of these medicinal products (acetaminophen, ethanol, theophhylline, enflurane, halothane, isoflurane), with CYP 3A4 subtracts (garlic can decrease the effectiveness of these drugs (calcium channel blockers, cancer drugs, ketoconazole, lidocaine…), with anticoagulants and antiplatelet drug (garlic has impact on the coagulation mechanism, it might slow blood clotting), with warfarin (garlic increase the effectiveness of warfarin).

FDA had adverse against the potential drug interaction of garlic capsules with saquinavir and does not recommended the co administration.

Studies

- Saquinavir (Piscitelli 2001)

This 2-treatments, 3-periods single sequence study was conducted in ten healthy volunteers 10 doses of saquinavir 1200 mg three time per day with meal for 3 days, then they received garlic caplets twice daily from D5 to D24, there was a new sequence of saquinavir, 10-day washout and the final period of saquinavir. PK analyses were done at the end of each saquinavir period. Garlic significantly decreased the Cmax and the AUC of saquinavir, but all patients did not have the same results. A control group with only saquinavir is lacking and it is difficult to extrapolate these results to other garlic formulations. Both galic and saquinavir are metabolised by CYP450.

- Ritonavir (Gallicano K. 2002)

A study has assessed the interaction between ritonavir (400 mg) and natural source odourless garlic (extract in oil), 2 capsules/day for 4 days. No significant change was observed, the AUC and the Cmax decreased respectively by 17 and 1%. This study is quite short to properly assess this possible interaction.

Two cases reports of HIV patients who took garlic for a long time when started ritonavir (400 or 600 mg) experimented severe gastrointestinal toxicity. The adverse event disappeared when one of the drugs was stopped. There is one positive rechallenge. The composition and the type of garlic were unknown. These events can be linked to high local concentration of garlic or ritonavir due to the inhibition of CYP3A4.

- Effects on CYP2D6 and 3A4 (Markowitz 2003)

- The influence of garlic (extract 3*600 mg twice daily) on CYP2D6 and 3A4 was assessed respectively with the coadministration of dextrometorphan (2D6 substrate) and alprazolam

(3A4 substrate). The study's duration was 14 days. The conclusions of this study were that garlic did not influence the pharmacokinetic of both components.

- Ciclosporine (Jabbari A 2005)

A clinical trial was conducted in 40 renal transplant patients. They received 1g of garlic by chewing or swallowing for 2 months, after that there is one month of wash out, the administration of the same dose of garlic by the other way. This study did not demonstrate a change in cyclosporine pharmacokinetic. The dose of cyclosporine was not known.

- Warfarin: garlic had impact on the coagulation mechanism and on platelet activity.

In a randomised, double-blind, placebo-controlled study by Macan (2006), conducted in 66 patients (only 48 completed the study), the effect of aged garlic extract (5ml twice daily for 12 weeks) on warfarine was assessed. There is no increased of haemorrhage in both groups and no modification of the INR in garlic arm.

In an open-label, three treatments (Warfarin 25 mg single dose, garlic tablets dosed at 2000mg and cranberry juice) study by Abdul (2008), garlic did not have any effect on platelet aggregation, pharmacokinetic, pharmacodynamic of Warfarin in healthy subjects.

- Docetaxel (Cox MC 2006):

The study was conducted in 10 women with metastatic breast cancer treated with docetaxel (30 mg/m²) for 3-4 weeks. Garlic was given twice daily at 600 mg in tablets from the third day after the first docetaxel dose and for 12 days. There is not control group in this study.

Garlic did not mainly change docetaxel pharmacokinetic except in patients with CYP3A5*1A allele.

Assessor's comment:

Several in vitro studies have assessed the effect of garlic on CYPP450 isoforms (Foster 2011, Greenblatt 2006, Ho 2010, Zhou L 2002). The study by Greenblatt concluded that garlic is unlikely to inhibit CYP450 isoenzymes. The study by Zhou demonstrated that allicin was a potent inhibitor of CYP2C9 and CYP 2C19 but not 1A2, 2D6, or 3A4. The study by Foster suggested that garlic may inhibit CYP 2C9, 2C19, 3A4 and 3A5.

However, in the rat, daily administration of garlic significantly increased hepatic CYP activity (Dhamija P 2006; Haber D 1994; Dalvi RR 1992).

These findings suggest that a long duration of garlic intake may decrease significantly drugs metabolized by CYP3A4 and are reinforced by results of the saquinavir study.

Therefore, although there are no formal DDI studies for the major drugs that are substrates of CYP3A4 (except for saquinavir), it is expected that long duration intake of garlic supplements may induces CYP 3A4 and as result, decrease plasma concentration and effects of drugs significantly metabolised by CYP3A4. Accordingly, the monograph should mention that garlic supplements may reduce the effect of oral contraceptives, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, cyclosporine etc.

In addition, considering the bleeding risk discussed in section 5.3, the monograph should advise that garlic supplements may increase bleeding times, and recommend using with caution garlic supplements with oral anticoagulation therapy and/or antipalelet therapy.

5.5.5. Fertility, pregnancy and lactation

According to ESCOP monograph, there are no objections to use garlic during pregnancy and lactation (because neither long-term nutritional experience nor any other important circumstances give reason for suspicion). From a controlled trial, it is known that major sulphur-containing volatiles from garlic are transmitted to human milk leading to improved drinking habits of the babies (Mennella and al, 1991).

WHO monograph consider that there are no objections to the use of Bulbus Allii Sativi during pregnancy and lactation, although excretion of the components of Bulbus Allii Sativi into breast milk and its effect on the newborn has not been established.

Maternal garlic ingestion has a reputation for causing colic in breastfed infants. Two papers tend to refute this claim. In one, 153 mothers who answered a questionnaire were no more likely to report colic in their infants in the previous week if they had ingested garlic than if they had not (Lust *et al.,* 1996). In another, mothers who were given either 1.5 grams of garlic or placebo capsules once daily in a blinded fashion for 3 days were asked if their infants had exhibited any signs of colic after capsule ingestion (were fussier, cried more or had more gas). Four of 20 women who ingested garlic thought their infants had colic; however, 4 of 10 women who received placebo thought they had received garlic and reported colic in their infants (Mennella *et al.,* 1993).

Assessor's comment

Available data are insufficient to establish the safety of Garlic preparations use during pregnancy and lactation.

5.5.6. Overdose

No data available

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

No data available

5.5.8. Safety in other special situations

Not applicable

5.6. Overall conclusions on clinical safety

Few adverse events were reported in clinical trials and case reports. The most common are gastrointestinal discomfort/pain and nausea, malodorous breath or body odour.

Regarding bleeding risk, in the absence of sufficient data, garlic consumption should be avoided 7 days before surgery, especially if high operative and postoperative bleeding risk. Patients taking oral anticoagulation therapy and/or antipalelet therapy should be recommend using garlic supplements with caution with such drugs.

6. Overall conclusions (benefit-risk assessment)

The review of the clinical studies data reported in this assessment report show that they were inconclusive to a well established use any indication.

Regarding traditional use, 2 situations were identified

- Prevention of atherosclerosis: the trends observed in these studies suggest that garlic supplementation may produce mild benefits on decreasing :

    o total cholesterol, triglycerides, and LDL-C levels

    o platelet activity,

    o blood pressure,

    o arteries stiffness

- The combination of these effects may support traditional use as an adjuvant for the prevention of atherosclerosis Preventing or treating the common cold: there is insufficient clinical evidence to grant an indication but data from two clinical trials (Josling, 2001 and Nantz 2012) may support traditional use in preventing or treating the common cold.

Garlic has been part of our diet for long time. We could consider that the oldness of its use may guarantee the use of large doses safely, however data form clinical trials and case reports highlight some of the adverse and toxic effects. Appropriate information regarding these adverse events should be provided to the users. Additionally, given the absence of sufficient clinical data, even though most of adverse event reported are mild to moderate, a special attention should be paid to bleeding risk in patients at high risk of bleeding.

Otherwise, appropriate information regarding DDI should be provided to users to insure a good safety use of garlic preparations.

In conclusion, due to its long-standing use and based on the available documentation, only a traditional use can be granted for garlic bulb. Only the preparations which have been used for at least 30 years including at least 15 years in the European Union are described in the monograph.

The monograph information should remain limited to the traditional use to "product used for the prevention of atherosclerosis associated with diet and exercice.

The use for the relief of the symptoms of cold.could also be mentioned as the product is considered as a traditional use since 1985 in Sweden and 1987 in UK.

As there are no clinical studies conducted with garlic bulb in children under the age of 12 years, garlic bulb should not be used in this target population and should be limited to adolescent over 12 years, adults and elderly.

Due to the lack of genotoxicity testing, a Community list entry cannot be established.

Annex

List of references