EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 March 2025
EMA/HMPC/329433/2024
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Allium sativum* L., bulbus

Draft - Revision 1

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)		*Allium sativum* L., bulbus (garlic)
Herbal preparation(s)		a) Powdered herbal substance b) Liquid extract from fresh bulb (DER 2-3:1), extraction solvent rapeseed oil, refined c) Dry extract (DER 5:1), extraction solvent ethanol 34 %
Pharmaceutical form(s)		Herbal preparation in solid dosage forms for oral use
First assessment	Rapporteur	J. Viguet Poupelloz
	Assessor(s)	Clinical: L. Boudali, D. Boucaud-Maitre Non clinical: E. Grangier
	Peer-reviewer	I. Chinou
Revision	Rapporteur	O. Palomino
	Peer-reviewer	I. 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 draft 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.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Table of contents

1. Introduction

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

- Herbal substance(s)

According to the European Pharmacopoeia (01/2019:1216), Garlic powder (Allii sativi bulbi pulvis) is defined as the Bulb of *Allium sativum* L., with the outer corneous layer removed, cut, freeze-dried or dried at a temperature not exceeding 65 °C and powdered.

Content: minimum 0.45 per cent of allicin ($C_6H_{10}OS_2$; Mr 162.3) (dried drug).

The composition of Allium bulbus is complex. Garlic contains volatile oil (0.1-0.36 %), the major components are sulfur compounds like alliin. It contains also proteins (amino acids, glutamyl peptides…), glycosides, 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).

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

Polyphenolic compounds such as apigenin, quercetin, nobiletin, tangeretin, rutin, allixin, myricetin and bergamottin (coumarin) from garlic have been determined. However, their content in raw and processed garlic is very low (Lanzotti, 2006).

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 identified (Lanzotti, 2006).

- Herbal preparation(s)

No pharmacopoeia monographs are available for preparations from *Allium sativum* L..

There is one preparation from garlic widely used: aged black garlic (ABG) is obtained after a treatment (sometimes called "fermentation") of the fresh garlic bulb at high temperature and controlled humidity for a period of weeks to several months. Garlic cloves become dark with a jelly-like consistency, and this process yields a sweet taste without the pungent off-flavour of fresh crushed garlic. The aged bulb undergoes substantial biochemical changes that distinguish it from fresh garlic. The bulb tends to lose water and releases free sugars (especially fructose), which combine with amino acids and appear to be key intermediate compounds of the Maillard reaction —furfurals and melanoidins —when browning. Soluble polyphenols and flavonoids also usually increase. However, the most important change is related to organosulfur chemicals. Garlic, as observed in other Allium species, fixes inorganic sulphate (SO42–) as L-cysteine and produces various organosulfur compounds with functional activity. Aging modifies the profile and quantities of those molecules. The main organosulfur compounds in fresh garlic (i.e., alliin and allicin) are downregulated by inactivation of alliinase and thermal degradation of the products. Polysulfides, such as diallyl sulfide, diallyl disulfide, diallyl trisulfide, dithiins, and ajoene, appear after decomposition of allicin. S-allyl-L-cysteine (SAC), which is virtually absent in fresh garlic, is synthetized and initially upregulated by deglutamylation of -glutamyl-S-allyl-cysteine and reaches several times higher concentrations than those in fresh tissues before its progressive catabolism to other compounds (Serrano *et al.,* 2023; Valls *et al.,* 2022).

Nonetheless, preparations with ABG do usually have different levels of SAC and many other components, and thus it is not always possible to compare them.

- 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

This Assessment Report resulted from the systematic review of that previously issued (EMA/HMPC/7686/2013) considering the new information from data published in the literature between 2017 and 2023.

Search engines used: Google, Google Scholar

Scientific databases: Web of Science; PubMed; Science Direct; Clinical Key; Cochrane Database of Systematic Reviews

Medical or Toxicological databases: Tox-line

Search terms: Allium sativum, garlic (1st January 2017- 30th October 2023).

Pharmacovigilance resources: Data from EU and non-EU regulatory authorities, European database for suspected adverse drug reaction reports. Search terms: Allium sativum.

Data from EU and non-EU regulatory authorities: Assessment report on *Allium sativum* L.*, bulbus* (EMA/HMPC/7686/2013)

Other resources: Interested parties provided information regarding new marketed products during the call for scientific data (30/04/2023 to 31/07/2023).

1.3. Main changes introduced in the first revision

In the first revision, the updated market overview revealed new products in the market with the same indication and similar or different posology than the ones included in the MO. The main changes introduced in this first version refer to the preparations included in the EU monograph: two marketed medicinal products already included in the original AR but not in the MO, do now fulfil the 30 years criteria: dried power for Indication 1) with a lower posology and dried powder for Indication 2), which is a new preparation for this indication.

In this revision, the information regarding the non-clinical and clinical studies assessed during the first version of the AR of *Allium sativum* L., *bulbus*, have been shortened, as no new outcome that could change the current MO content is possible.

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

Information on medicinal products marketed in the EU/EEA

Garlic bulb as single herbal substance is authorised in several member states: Denmark, France, Germany, Hungary, Latvia, Poland and Spain.

Table 1: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Strength (where relevant)** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
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 generalized arteriosclerosis	200mg. 2 tablets 3 times daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalized arteriosclerosis	300mg. 1 tablet 3 times daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalized	210 mg. 2 tablets 3 times daily	At least since 1976 Germany

Active substance	**Indication**	**Pharmaceutical form** **Strength (where relevant)** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
	arteriosclerosis		
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalized arteriosclerosis	300 mg. 1 tablet four times daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalized 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 generalized arteriosclerosis	230 mg. 2 tablets 3 times daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalized arteriosclerosis	250 mg. 5 tablets daily	At least since 1976 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalized arteriosclerosis	330 mg. 1 soft capsule 3 to 4 times daily	1995 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalized arteriosclerosis	100 mg. 3 tablets 3 times daily	1996 Germany
Dried powder	For oral use, in adults and adolescents: prophylaxis of generalized arteriosclerosis	200mg. 2 tablets 3 times daily	1999 Germany
Dried powder	For oral use, in adults and adolescents:	300 mg. 1 tablet 3 times daily	1999

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
	prophylaxis of generalized arteriosclerosis		Germany
Dried powder	For oral use 1) Adults: for the prevention of atherosclerosis, in cases of mild hypercholesterolemia, prevention of changes in blood vessels occurring with age 2) 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 generalized 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 generalized 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 % alliin, equivalent to 0.6 allicin. 1 dragee 3 times daily	1992 Hungary

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
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 in arteriosclerosis prophylaxis, un mild forms of hyperlipidaemia and hypercholesterolemia, in prevention of old-age vessels changes and in the early stage of infection of the upper respiratory tract	300 mg 1-2 tablets 2-3 times a day In the case of infection of the upper respiratory tract: 2 tablets 3-4 times a day	1990 Poland
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 cold	100 mg corresponding to 500 mg fresh bulb. 1 to 2 tablets 1 to 2 times daily	1985 - 2014 Sweden
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** **Strength (where relevant)** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
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 (where relevant)

In Italy, Food supplements are on the market with different health claims: 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

Garlic bulb as single herbal substance is authorised in the United Kingdom since 1990: Dried powder is marketed for oral use in adults and children over 8 years for the relief of catarrh and rhinitis (150 mg, 3-daily for adults and 2 tablets daily for children over 8 years).

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. *Allium sativum* is probably indigenous to Asia but it is commercially cultivated in most countries (WHO 1999) and is clearly one of the most popular herbal remedies worldwide today.

Garlic is cultivated around the world, from Mediterranean climates to Siberia. Ancient Egyptians used it as a form of currency. 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, 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, 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 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, 2000).

Arabian herbalists used 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, 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, 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, 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, 2000). It has also been used for chronic bronchitis, asthma, and whopping cough (Steinegger & Hänsel, 1972).

Allium sativum has been used as fresh bulb, infusion, juice or tincture for many therapeutic uses including asthma, scurvy, pinworm, cholera, typhus, chronic bronchitis, diphtheria and influenza; also has been used as antiseptic, expectorant, diaphoretic and for the prevention or treatment of arteriosclerosis (Madaus, 1979).

The WHO-Monograph (1999) includes its use 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.

According to the ESCOP monograph (2003), *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 adequate clinical data to support this indication are not available.

In the British Herbal Pharmacopoeia (BHP) (1976 and 1983), *Allium sativum* is indicated against chronic bronchitis, respiratory catarrh, recurrent colds, whooping cough, bronchitic asthma and influenza. The preparations can be used in children, but only small doses diluted should be administered. In the BHP, 1990, it is reported as hypolipidaemic and antimicrobial, with no specified posology.

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 (Martindale, 1972). The preparations of the garlic juice and the garlic syrup were described in the British Pharmacopeia Codex (1949).

Table 3: Overview of historical data

Herbal preparation	Documented Use / Traditional Use	Preparation	Reference
a) fresh garlic b) dried powder c) oil d) Other preparations	As an adjuvant to dietetic management in the treatment of hyperlipidaemia, and in the prevention of atherosclerotic (age-dependent) vascular changes. Treatment of respiratory and urinary tract infections, ringworm and rheumatic conditions. Used as a carminative in the treatment of dyspepsia	a) 2–5g b) 0.4–1.2 g c) 2–5mg d) Other preparations should correspond to 4–12mg of alliin or about 2–5mg of allicin). Bulbus Allii Sativi should be taken with food to prevent gastrointestinal upset.	1999 WHO monograph
a) Clove b) Powder c) Dried bulb d) Tincture (1:5, ethanol 45 %)	a) et b) prophylaxis of atherosclerosis or treatment of elevated blood lipid level c) 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

The information about therapeutic indications of preparations from garlic is available from literature and from the market overview which reveals a continuous use for more than 30 years in the EU.

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…).

Table 4: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
Dried powder. Hard capsules	Traditionally used in the treatment of	430 mg. 3 capsules daily (1290 mg)	1981

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
	minor circulatory disorders		France
Dried powder. Tablets	Prophylaxis of generalized arteriosclerosis	200mg. 2 tablets 3 times daily (1200 mg)	At least since 1976 Germany
Dried powder. Tablets	Prophylaxis of generalized arteriosclerosis	300mg. 1 tablet 3 times daily (900 mg)	At least since 1976 Germany
Dried powder. Tablets	Prophylaxis of generalized arteriosclerosis	210 mg. 2 tablets 3 times daily (1,26g)	At least since 1976 Germany
Dried powder. Tablets	Prophylaxis of generalized arteriosclerosis	300 mg. 1 tablet four times daily (1,2 g)	At least since 1976 Germany
Dried powder. Tablets	Prophylaxis of generalized arteriosclerosis	250 mg. 5 tablets daily (2 after lunch, 3 after dinner) (1,25g)	At least since 1976 Germany
Dried powder. Tablets	Prophylaxis of generalized arteriosclerosis	230 mg. 2 tablets 3 times daily (1,38g)	At least since 1976 Germany
Dried powder. Tablets	Prophylaxis of generalized arteriosclerosis	250 mg. 5 tablets daily (1,25g)	At least since 1976 Germany
Dried powder	Traditionally used for mild cardiovascular problems	330 mg. 1 to 2 hard capsules 2 times daily (660 mg)	1987 Spain
Dried powder	Prevention and complementary treatment of atherosclerosis, for the relief of cardiovascular disturbances deriving from atherosclerosis	100 mg corresponding to 1.3 % alliin, equivalent to 0.6 allicin, 3 times daily	1992 Hungary
Dried powder	1) Traditionally in arteriosclerosis prophylaxis, un mild forms of	1) 300 mg 1-2 tablets 2-3 times a day (1800 mg)	1990 Poland

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
	hyperlipidaemia and hypercholesterolemia, in prevention of old-age vessels changes 2) Early stage of infection of the upper respiratory tract	2) 300mg 2 tablets 3-4 times a day (2400mg)	
Extract from fresh Allii sativi bulbus (DER 2-3:1), extraction solvent Rapae oleum raffinatum. Gastro-resistant capsules, soft	Traditional used for prophylaxis of generalized arteriosclerosis	108 mg. 1 to 2 capsules 4 times daily (432-864mg)	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 (200-400mg)	1985 - 2014 Sweden

Historical data and documented period of use in the EU support the evidence of traditional use of different preparations of garlic for:

Indication 1) as an adjuvant for the prevention of atherosclerosis. *Adults and elderly.* Powdered herbal substance and Liquid extract from fresh bulb (DER 2-3:1), extraction solvent rapeseed oil, refined

and

Indication 2) for the relief of the symptoms of common cold. *Adolescents, adults and elderly*. Powdered herbal substance and Dry extract (DER 5:1), extraction solvent ethanol 34% V/V.

3. Non-Clinical Data

The antiplatelet and antioxidant effects of garlic seem to be related to allicin and its transformation products (ESCOP, 2003).

The literature reported several references of non-clinical data for garlic and its constituents. Some controversial results have been obtained, probably due to the different constituents of garlic or garlic preparations used and the different durations and design of the study.

Only some non-clinical references are mentioned in this AR in order to support the main garlic effects.

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

3.1.1. Primary pharmacodynamics

Lipid-lowering effects

Garlic preparations have shown beneficial effects on the levels of cholesterol and plasma lipids, lipid metabolism and atherogenesis both *in vitro* and *in vivo*.

In vitro studies

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 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).

In vivo studies

Anti-hypercholesterolemic 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 *et al*., 2000).

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

Antihypertensive effect and effect on vascular resistance

In vitro studies

Garlic extracts elicited a marked vasorelaxant effect, which depends, in part, on the synthesis and release of NO (Das et al, 1995; 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).

In vivo studies

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

An aqueous garlic extract administrated 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).

Effect on platelet aggregation

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

In vitro studies

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).

In vivo studies

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).

Table 5: Overview of the main non-clinical data/conclusions

Herbal preparation tested	**Posology**	**Experimental model**	**Reference**	**Main non-clinical conclusions**
comparable/similar preparations to preparations of the monograph				
Fresh garlic, Garlic extract, garlic oil	Oral or intragastric administration	*In vivo* in rats,rabbits, chickens, pigs	Ismail *et al*, 1999	Anti-hypercholesterolaemic and anti-hyperlipidaemic effects
Garlic powder	Oral administration, 50mg/kg	*In vivo* in Hypercholesterolemic rats	Ali *et al.,* 2000	Reduction in blood cholesterol and triglycerides levels during 6 weeks period
Garlic powder	Oral administration (0.5% in diet)	*In vivo* in Hypertensive rats	Brandle *et al*., 1997	Significant decrease in systolic blood pressure
other preparations				
Aqueous extract of garlic, not further specified	Oral or intraperitoneal administration, 50 mg/kg	*In vivo* in Rats	Bordia *et al,* 1996	Significant inhibition of thromboxane B2
Aqueous garlic extract not further specified	Oral administration, single or repeated doses at 50 mg/kg body weight for 2 weeks	*In vivo* in Rat two-kidney-one-clip Goldblatt model (hypertensive rats)	Al-Qattan, 1999	Significant antihypertensive effect
single substances				

3.1.2. Secondary pharmacodynamics

Hypoglycaemic effect

Hypoglycaemic effects of garlic preparations and its constituents have been demonstrated *in vivo* 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).

Antioxidant activity

The antioxidant effect of garlic preparations was able to prevent 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 studies performed in vivo and in vitro (Kabasakal et al, 2005).

The antioxidant effect of an aqueous extract obtained from 1 mg of the garlic preparation was found to be 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)

Antimicrobial activity

The antibacterial and antifungal activity of garlic is described in the literature (WHO, ESCOP): different 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 data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

Non-clinical data are mainly coming from *in vitro* studies. These studies and the published results in animal models are not able to explain the mechanism of action of garlic powder in the claimed indications. Although some investigations suggest that the biological and medical functions of garlic are

mainly due to their high organo-sulfur compounds content (Omar et al, 2010), none of the published studies do support the therapeutic use of the drug.

Data on safety pharmacology and pharmacodynamic interactions are not available.

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

Herbal substance

No pharmacokinetic data about garlic preparations are available.

Relevant constituents

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 unmetabolized 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 oral 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

In vitro studies

An *in vitro* study was performed to ascertain the potential risk 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 metabolized 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).

Another *in vitro* study was performed in immortalized 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).

In vivo studies

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.

A study was conducted in rats to assess the pharmacokinetic interaction between propranolol (10mg/kg p.o.) and fresh garlic extract (250 mg/kg p.o.). 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 (Asdaq *et al*. 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

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

The Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) re-evaluated the safety of Garlic Oil for use as flavoring in food, as part of a series of evaluations of natural flavor complexes (NFCs) for use as flavoring ingredients. The constituents each NFC were organized into well-defined congeneric groups and the estimated intake of each constituent congeneric group was evaluated using the conservative threshold of toxicological concern (TTC) concept. Based on the safety evaluation, garlic oil was affirmed as generally recognized as safe (GRASa) under their conditions of intended use as flavor ingredients (Davidsen *et al.,* 2023).

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 summarized in Table 6.

Table 6: 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 (*i.v.*)	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 (p.o.)* Intraperitoneal Subcutaneously	LD50 > 30 ml/kg
Mills and Bone 2005	Aqueous extract of crushed fresh garlic	Rat	Undefined	LD50 = 173.8 ml/kg
Nwanjo and Owo 2007	Aqueous garlic extract	Rat (6 rats/group)	Intraperitoneal (*i.p.*) 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 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 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	Garlic oil feeding (10 mg/100 g body wt, intragastrically) after 24 hr fasting was found lethal (all died :6/6): - cause of death appears to be acute pulmonary oedema - all organs revealed severe congestion on the histopathological examination. - the liver showed histological changes
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,	Allicin	Mouse	Intravenous	LD50 = 60 mg/kg

2003)				
Cavallito, 1944 (cited in ESCOP, 2003)	Allicin	Mouse	Subcutaneous	LD50 = 120 mg/kg
Mills and Bone 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 (DAS)	Mouse	Intraperitoneal	LD50 = 500 mg/kg Results indicated that lungs, liver, and reproductive organs were affected at higher DAS doses and thus, excessive usage of DAS should be limited owing to the associated toxicities at higher concentrations (1600 mg/kg and above).
Dutta et al. (2021)	Diallyl sulfide (DAS)	Mice	Intraperitoneal (160, 1280, 1600, and 1920 mg/kg)	A single dose of DAS up to 1280 mg/kg was well-tolerated without significant changes in standard toxicological parameters. Death was observed at 1600 mg/kg (1/5 male) and 1920 mg/kg (2/5 female and 3/5 male) doses. DAS at 1920 mg/kg dose level also induced marked pathological changes in the lungs, liver, and reproductive organs.

Assessor's comment

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

The study performed by Joseph et al (1989) with garlic oil suggested a warning with garlic oil but at the same time, similar feeding of garlic oil was well tolerated by rats in the fed state. Also, 24 hr fasted rats could tolerate this dose of garlic oil, provided they were previously adapted to garlic oil feeding. Some otherstudies performed with garlicextracts are reassuring, including the study performed by Mikail et al (2010) with an aqueous extractby subcutaneous route which showed a LD_{50} value of 3034 mg/kg. This low value is an indicator of relatively safe and reduced risk of toxicity. A more recent safety evaluation confirmed garlic oil as generally recognized as safe (GRAS) under the conditions of intended use as flavor ingredients (Davidsen et al., 2023).

3.3.2. Repeat dose toxicity

The toxic effects were studied and reported in references from 1939 to 2023. Garlic powder, fresh garlic, garlic juice and garlic extracts were evaluated in rat and guinea pig.

Garlic

The available data are summarized in Table 6.

Two 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 concluded 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.

Garlic oil

The 28-day repeated dose oral toxicity study of garlic essential oil (GEO) was studied in mice by Lin *et al.*, (2022). A repeated dose of GEO (15, 25, and 50 mg/kg body weight, p.o.) was administrated to ICR mice for 28 days to ascertain the subacute toxicity of GEO.

Results showed that GEO did not affect the ratio of immature to total erythrocytes or the number of micronuclei in immature erythrocytes of ICR mice after 24 and 48 h. In a 28-day oral toxicity assessment, GEO (15, 25, and 50 mg/kg body weight, p.o.)-fed ICR mice exhibited normal behaviors, mortality, body weight, daily intake, hematology, clinical biochemistry, and organ weight. According to these data, the no-observed-adverse-effect level (NOAEL) for GEO is considered to be greater than 50 mg/kg bw/day orally for 28 days in mice.

Isolated compounds

Diallyl sulfide

Dially sulfide (DAS) is an important component in garlic oil. Dutta *et al*. (2021) investigated the pre-clinical toxicity of DAS to identify the maximum tolerable and lethal doses and understand its underlying mechanisms at toxic doses.

DAS was administered intraperitoneally to C57BL/6 mice at a range of concentrations (160, 1280, 1600, and 1920 mg/kg). Standard toxicological endpoints (survival, body weight changes, hematology, histopathology, and serum biochemistry) were supported with genotoxicity and molecular studies.

A single dose of DAS up to 1280 mg/kg was well-tolerated without significant changes in standard toxicological parameters. Death was observed at 1600 mg/kg (1/5 male) and 1920 mg/kg (2/5 female and 3/5 male) doses. DAS at 1920 mg/kg dose level also induced marked pathological changes in the lungs, liver, and reproductive organs. This dose increased hepatic enzyme activities and significantly reduced blood cell count. Inflammatory markers like CD68 and NF- κB were upregulated in the lungs. Propidium iodide stained ovarian and testicular germ cells revealed alterations in cell cycle distribution and increased cell death at 1920 mg/kg dose.

Results indicated that lungs, liver, and reproductive organs were affected at higher DAS doses and thus, excessive usage of DAS should be limited owing to the associated toxicities at higher concentrations (1600 mg/kg and above).

Table 7: 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 <u>After 45 days of treatment:</u> degenerative changes in testes (seminiferous tubule and leydig cells nuclei were shrunken) but normal stages from spermatogonia to spermatids <u>After 70 days of treatment:</u> - Severe testicular lesions - Spermatogenesis arrest at the primary spermatocyte stage - Sertoli cells degenerative changes
Carl, 1939 (cited in FDA	Fresh garlic	Rat (5)	- duration not known	*(endpoint not detailed)* All animals died within 11 days

evaluation for the GRAS status, 1973)			- oral route 20 to 30 % in diet	
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)* <u>Body and organ weights</u> - ↓ bodyweight - ↓ seminal vesicle and prostate weight <u>Hormonal measurements</u> - ↓ serum testosterone level with ↑ LH concentration <u>Biochemistry on accessory gland</u> - ↓ prostate citric acid - ↓ seminal vesicles fructose <u>Testicular analysis:</u> - 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, CypI Ia, HSD3b5 and Hsd17b)
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, haemorrhage, 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-oxidant, 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 antioxidants catalase (CAT) et SOD) – 1000mg/kg/day: histopathological and ultrastructural changes in liver (large aeras of necrosis with haemorrhage 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	Wistar rat	- 6 months - oral route 60, 200, 600 and	*(endpoints: body weight, clinical pathology, histopathological examinations (organs and tissues not detailed):* No toxic signs

	specified)		2000 mg/kg 5 times a week	
Dutta et al., 2021	DAS	C57BL/6 mice	160, 1280, 1600, 1920mg/kg i.p.	Pathological changes in lungs, liver, reproductive organs Increased hepatic enzyme activity, inflammatory markers and alterations in cell cycle distribution at 1920mg/kg
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 but became normal after 2 months
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 juice targeted generally liver and kidneys and occasionally stomach. Even if the findings suggested liver and kidneys are target organs, it must be shown that no study was 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 (Jubb, 1947). The studies are very old and not well-documented, the duration is not known, and the endpoints are not detailed.

The studies 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 is reported in section 5.3.

More recent studies indicated that high doses of DAS affected lungs, liver, and reproductive organs and thus, excessive intake of DAS should be limited owing to the associated toxicities at higher concentrations (1600 mg/kg and above) (Dutta et al., 2021).

3.3.3. Genotoxicity

The genotoxic potential was studied and reported in references from 1984 to 2023. Garlic powder, garlic extracts and its constituents (diallyl sulfide and diallyl disulfide) were evaluated for genotoxicity and mutagenic potential in the bacteria and mammalian microsome mutagenicity test and for chromosomal aberrations induction in *in vivo* experiments.

Garlic oil

Despite the fact that garlic is considered to have very low toxicity and is listed as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration, some studies assayed the genotoxic pitential of garlic oil (Joseph et al. 1989).

The genotoxicity of garlic oil (GEO) was studied by Lin *et al.,* (2022). Ames test using five *Salmonella typhimurium* strains (TA98, TA100, TA102, TA1535, and TA1537) and Chinese hamster ovary (CHO-K1) cells with or without metabolic activation (S9 system), and mammalian erythrocyte micronucleus test were used to assess the genotoxicity and clastogenic effects of GEO. The results of the Ames test with or without S9 system indicated that GEO did not induce mutagenicity nor have clastogenic effects in CHO-K1 cells with or without S9 activation.

Isolated compounds

Propyl propane thiosulfinate (PTS)

The study conducted by Mellado-García *et al.*, (2017) was aimed to assess the cytotoxicity, mutagenicity and genotoxicity of an organosulfur compound (OSC) from a garlic extract. Propyl propane thiosulfinate (PTS) is an OSC present in garlic with antimicrobial activities.

The bacterial reverse-mutation assay in *S. typhimurium* (Ames test, OECD 471, 1997), the micronucleus test (MN, OECD 487, 2016), the mouse lymphoma thymidine-kinase assay (MLA, OECD 476, 2015), and the comet assay (standard and modified with restriction enzymes) were performed to test the toxicity of PTS.

Results revealed that PTS was not mutagenic neither in the Ames test (in the presence/ absence of S9) nor in the MLA (after 4 and 24 h of exposure) at any of the tested concentrations. However, genotoxic effects were recorded for the MN assay in L5178Y Tkþ/- cells in both absence and presence of S9. Similarly, positive results were also found in the comet assay at the highest concentration tested (280 mM) in Caco- 2 cells after 24 and 48 h of exposure. However, any DNA-oxidative damage (by using the Endo III and FPG-modified comet assay) was observed. A substance is only considered negative for genotoxicity if all tests performed are assessed negative. According to the obtained results further *in vivo* genotoxic studies were recommended in order to confirm the genotoxic profile of PTS.

Diallyl sulfide (DAS)

Dutta *et al.* (2021) investigated the genotoxicity of DAS which was administered intraperitoneally to C57BL/6 mice at a range of concentrations (160, 1280, 1600, and 1920 mg/kg). At the highest dose of DAS, evidence of genotoxicity in terms of the occurrence of a few double minutes, rings, and fragments appeared. Authors found that reproductive organs were affected at higher DAS doses and concluded that excessive usage of DAS should be limited owing to the associated toxicities at higher concentrations (1600 mg/kg and above).

The available data are summarized in Table 8.

Table 8: Summary of genotoxicity studies

Ref.	Formulation	Type of test	Test system	Concentrations, metabolising system	Results
Garlic preparations					
Abraham and Kesavan, 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
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	– Mouse, micronuclei in peripherical	– 2.5, 5 and 10 mg/kg – 5 wistar albino male mice	**-** Equivocal test response: Garlic drops showed a significant dose-

		vivo	blood	– 2 administrations at 24h intervals (route not specified)	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
Mellado-García et al., 2017	Propyl propane thiosulfinate (PTS)	Bacterial reverse-mutation assay Micronucleus test (MN, OECD 487, 2016) Mouse lymphoma thymidine-kinase assay (MLA, OECD 476, 2015) Comet assay (standard and modified with restriction enzymes)	– *Salmonella typhimurium*	– PTS: 50-500 µM	- No mutagenicity - Genotoxic effects for MN assay in L5178Y Tkþ/- cells in both absence and presence of S9. - Positive results in the comet assay at the highest concentration tested (280 mM) in Caco- 2 cells after 24 and 48 h of exposure. - No DNA-oxidative damage (by using the Endo III and FPG-modified comet assay)
Dutta et al., 2021	Diallyl sulfide (DAS)	Genotoxicity	– Mice, *i.p.*	– 160, 1280, 1600, and 1920 mg/kg	- Genotoxic at 1920mg/kg: a few double minutes, rings, and fragments appeared - Reproductive organs affected

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.

Some genotoxicity studies have been performed with different preparations of garlic or isolated compounds. Diallyl sulfide (DAS) at high doses showed genotoxicity in terms of the occurrence of a few double minutes, rings, and fragments appeared; while also, reproductive organs were affected. Thus, excessive usage of DAS should be limited, and appropriate safe dosage of garlic oil need to be followed.

3.3.4. Carcinogenicity

No data available.

3.3.5. Reproductive and developmental toxicity

No data 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 adequate reproductive toxicity 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 data available.

3.3.7. Other studies

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

3.3.8. Conclusions

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 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 (Jubb, 1947).

More recent studies published by Dixit and Joshi (1982) and Hammami et al (2008; 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.

Genotoxicity studies with isolated components (PTS, DAS) showed toxic effects af high concentrations or doses (Mellado García *et al.,* 2017; Dutta *et al.,* 2021).

3.4. Overall conclusions on non-clinical data

Numerous *in vitro* and *in vivo* (rats, mice, rabbits and dogs) studies have been published with garlic powder, different extracts and with some isolated constituents of garlic. Experiments on lipids, on blood pressure, on platelet aggregation and antioxidant 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.

Results from relevant experimental studies on garlic powder to support the proposed indications are very limited. The reported pharmacological effects are not considered contradictory to the traditional uses.

Specific data on pharmacokinetics and interactions are not available.

Non-clinical information on the safety of garlic powder is scarce.

The acute toxicity of garlic powder remained unknown. Studies are very old and not well-documented, the duration is not known and the endpoints are not detailed (Jubb, 1947).

Studies on Reproductive Toxicity in accordance with ICH S5 (R3) are not available. From the few published studies, a potential impact on male fertility cannot be excluded. This finding will be reported in section 5.3 of the monograph.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

Oral administration of garlic powder can be regarded as safe at traditionally used doses.

Adequate tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed with garlic preparations or isolated constituents.

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

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 summarized in Table 8 (Amagase, 2006).

Table 9: Main type of products and characteristics of garlic products on the market

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

	Well-established safety Heavily researched (4001 papers)

* Allicin is a highly unstable and reactive metabolite 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).

Sulfur-containing compounds in commercial garlic preparations vary, depending on their manufacturing processes.

Antiatherogenic and lipid-lowering effects

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.

Hypoglycaemic properties

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-sulfuric compounds

The pharmacokinetic destiny of organo-sulfuric 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 exalted (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 glutamyl-cysteines, and (2) cysteine sulfoxides. Allyl-cysteine 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:

Allicin

Several studies have revealed that the bioavailability 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 transient compound that is rapidly decomposed to other products.

Flavonoids

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

Sapogenins and saponins

There is 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)

During this revision, 33 new metanalyses not yet available during the first assessment were identified. These publications reviewed the use and effect of garlic preparations in the treatment of metabolic syndrome, cardiovascular disease risk factors, atherosclerotic process, diabetic retinopathy, cough and cold, among others. Nonetheless, the most recent studies were performed with aged garlic extracts, a preparation which is not marketed as a medicinal product in any member state and so, not included in the EU monograph, while some publications include other different extracts not fully described.

49 new randomized clinical trials included the use of garlic preparations for different purposes such as metabolic syndrome, cardiovascular disease risk factors, atherosclerotic process and diabetic retinopathy. Most of them were performed in non-EU countries, some of them were related to indications/symptoms/diseases not included in the current MO (i.e., wound healing) and some of them were performed with garlic preparations not fully described or not included in the market overview of garlic medicines in the EU (i.e. aged black garlic).

This assessment report reflects only those studies relevant for the *Allium sativum bulbus* monograph.

Assessor's comments

Several clinical trials have been performed with aged garlic extracts for the treatment of different symptoms related to cardiovascular disease (Wlosinska et al., 2021), such as hypertension (Serrano et al., 2023), hypercholesterolemia (Valls et al., 2022) or atherosclerotic process (Wlosinska et al., 2020). A different garlic extract, not fully described, has also been assayed for its effect on markers of endothelial function in obese patients (Szulinska et al., 2018).

Nonetheless, and even though results suggested a positive outcome, all these studies have been carried out with different preparations than those covered by the monograph or are not fully described and thus, they cannot be taken in account for the assessment of the efficacy of garlic preparations in the cited conditions.

1. Antilipidemic effects

Clinical studies of good quality design and well conducted did not show any significant effects on LDL-cholesterol or other plasma lipid concentrations in adults with moderate hypercholesterolemia (Gardner, 2007) or results were not analysed on the intention-to-treat base (Mader, 1990).

Several meta-analysis and reviews have been published regarding the effectiveness and beneficial properties of garlic on cholesterol and triglycerides (Banerjee et al, 2002; Alder et al., 2003; Reinhart et al; 2009; Khoo et al., 2009; Tsai et al., 2012, Zeng et al., 2012, Ried et al., 2013). Nevertheless, the results of the meta-analysis on lipid levels are conflicting. While the old meta-analysis published before 2000 suggested an effect on lipid parameter, the more recent reviews, published between 2001 and 2012, have suggested a more modest role for garlic on plasma lipid levels or no effects.

In the review by Khoo et al. (2009) and given the substantial clinical heterogeneity of the populations enrolled (hypercholesterolemic 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 is not appropriate. Moreover, the authors concluded that the available evidence from randomized controlled trials did not demonstrate any beneficial effects on serum cholesterol. A separate analyse was conducted for comparisons of garlic preparation in healthy and hypercholesterolemic subjects showing similar results.

Only two trials (Zhang 2001; 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-lipidemic 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.

Moreover, there is no comparative study with accepted pharmacotherapy for the treatment of hypercholesterolemia.

Results on total cholesterol

Meta-analysis of 37 trials revealed that old trials seem to show a benefit on total cholesterol whereas more recent trials show more controversial results.

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. Subgroup analysis by effect of garlic type on LDL cholesterol was significant for garlic powder but not for aged garlic extract.

In patients with primary hypercholesterolemia, reduction in LDL-cholesterol could be considered as the primary endpoint to support the indication of hypercholesterolemia or mixed hyperlipidaemia. Nonetheless, 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 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.

Conclusion on the anti-lipidemic effects of garlic

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, reviews and trials published between 2002 and 2023, reported a more modest role for garlic on plasma lipid levels or no effects.

The diverse composition and amount of active sulfuric 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 emphasize the need for standardization 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 content 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.

The majority of the most recent clinical trials 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 lack of data

regarding the long-term maintaining effect, no well-established use could be granted in hypercholesterolemia (primary and mixed).

2. 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, 2007).

One meta-analysis of the effect of Bulbus Allii Sativi on blood pressure has reviewed a total of 11 randomized, controlled trials (published and unpublished) (Silagy et al., 1994i). 8 trials 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).

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.

The meta-analysis by Ried et al., 2008 analysed 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.

Eleven out of twenty-five 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 ($n = 3$; $p < 0.001$):

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: $R = -0.315$; $p = 0.02$).

In the revision by Simons *et al.,* 2009, the authors made a review on the influence of trial quality on the effect of garlic on blood pressure and concluded 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).

The analysis of the 5 clinical trials with the highest methodological score revealed no effect of garlic on blood pressure in normotensive subjects.

The Cochrane meta-analysis aimed to determine whether the use of garlic as monotherapy, in hypertensive patients is able to lower the risk of cardiovascular morbidity and mortality compared to placebo (Stabler, 2012).

The search identified two randomized 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 randomized 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 concluded 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.

The aim of the trial by Ried (2010) was 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. It 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.

The results showed that 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 $\geq$ 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.

The study by Ried (2013) was 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 (240/480/960 mg containing 0.6/1.2/2.4 mg of S-allyl-cysteine) or placebo. Mean systolic blood pressure was significantly reduced by 11.8±5.4 mm Hg 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±4.1 mm Hg, P=0.07). Changes in systolic blood pressure in the garlic-1-capsule group and diastolic blood pressure were not significantly different to placebo.

Table 10: Clinical studies on humans, in circulatory disorders

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Influence of gender in the effect of garlic oil on cholesterol and glucose levels Zhang, 2001	Randomized placebo-controlled trial 11 weeks	Garlic oil, 8.2mg/day Separate group, garlic powder, 1g/day	51 subjects	Healthy normo-lipidemic volunteers	Primary: change in plasma values of cholesterol	t-test of garlic effect by gender interaction CI 95%	No clinical relevance: no significant difference between groups, low number of (healthy) subjects Unexpected and analyses of gender effects not planned in advance.
Effect of raw garlic *vs* commercial garlic supplements on Plasma Lipid concentrations Gardner, 2007	Parallel-design trial 6 months	4 arms: raw garlic, powdered garlic, aged garlic extract or placebo. Garlic product doses equivalent to an average-sized garlic clove were consumed 6 d/wk for 6 months.	192 adults	Adults with LDL-C concentrations of 130 to 190 mg/dL (3.36-4.91 mmol/L)	Primary: LDL-C concentration. Fasting plasma lipid concentrations were assessed monthly	The minimal clinically significant between-group difference in LDL-C change selected was 10 mg/dL (0.03 mmol/L),	No clinical relevance: no statistically significant effects on HDL-c, TG or total cholesterol/ HDL-c ratio

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
						and a 20-mg/dL (0.52-mmol/L).Tthe study was powered for a moderate effect size of d = .5. Repeated measures taken over time were assessed using random effects regression models.	
Efficacy of garlic in hypertension and hyperlipidaemia Auer *et al*., 1990	Randomised, placebo-controlled, double-blind trial 12 weeks	Garlic powder (standardized as to 1.3% alliin)	47 subjects	Hypertensive patients	Blood pressure and plasma lipids	95% CI	Significant reduction of mean supine systolic and diastolic blood pressures *vs* placebo. No clinical relevance:

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
							i.e. short duration, low number of patients, garlic preparation not fully described
Effect, tolerability and acceptability of aged garlic extract in patients with treated, but uncontrolled, hypertension Ried, 2010	Double-blind parallel - randomized placebo-controlled trial 12 weeks	Four capsules of aged garlic extract (960 mg containing 2.4 mg S-allylcysteine) daily for 12 weeks	50 patients	Hypertensive patients	Primary: SBP and DBP	ITT yes	SBP including all participants did not reveal a significant difference between the groups from baseline to No significant differences of blood pressure in patients with SBP<140 mmHg at baseline. No clinical relevance

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Effect of aged garlic extract in hupertensive patients: dose response study Ried, 2013	Double-blind, randomized, placebo-controlled 12 weeks	Aged garlic extract: One, two or four capsules daily of aged garlic extract (240/480/960 mg containing 0.6/1.2/2.4 mg of S-allylcysteine) or placebo.	79 patients	Uncontrolled hypertension (SBP>140)	Primary: SBP and DBP	ITT yes	Mean SBP significantly reduced in the garlic-2-capsule group

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

Children

Cardiovascular effects

McCrindle *et 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, randomized, 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 randomized controlled trial comparing tablets of garlic to benzimidazole or placebo in children has been published (Andrianova et al. 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 randomized 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 by (Andrianova et al. 2003) is written in Russian and based only to the conclusions of the abstract, seems that garlic has no side effects. Data are insufficient to recommend precise dosages on paediatric population.

Assessor's conclusions on use of garlic in children

A paucity of good evidence 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 used as food and that garlic is approved in children in many countries (UK, Poland SE), garlic could be used with not problems 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.

- Anti-lipidemic 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 randomized 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 (hypercholesterolemic patients and healthy subjects). The separate analysis conducted for comparisons of garlic preparation in healthy and hypercholesterolemic 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 emphasized 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.

Updated meta-analysis of Ried in 2016 did not provide new evidence regarding effects on serum cholesterol and 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 sulfuric 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; Xiong and 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. In conclusion, available data are insufficient to grant an indication for hypertension.

However, the trends observed in these studies 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.

The combination of these effects is in line with the traditional use as an adjuvant for the prevention of atherosclerosis

- Antithrombotic effect / Cardiovascular morbidity and mortality

The in vivo anti platelet seems established, however 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 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 regarding this indication is available.

- Prevention and treatment of symptoms of common cold

Regarding the prevention or treatment of the common cold, even though the Cochrane meta-analysis (Lissiman *et 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 et al., 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.

- Hypoglycaemic properties

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.

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. On the basis of long-standing use and experience, the plausibility of efficacy of *Allium sativum* L., *bulbus* as an adjuvant for the prevention of atherosclerosis can be accepted.

Regarding the prevention or treatment of the common cold, the Cochrane meta-analysis (Lissiman et al., 2012) concluded 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 et al. 2001) showed fewer days of illness in the garlic group compared with the placebo group. Moreover, another recent trial (Nantz et al. 2012) suggested that consuming the aged garlic extract could reduce severity of symptoms reported. Therefore, on the basis of long-standing use and experience, the plausibility of efficacy of *Allium sativum* L.*, bulbus* for prevention or treatment of symptoms of common cold can be accepted.

5. Clinical Safety/Pharmacovigilance

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

Table 11: Clinical safety data from clinical trials

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
Influence of gender in the effect of garlic oil on cholesterol and glucose levels Zhang, 2001	Randomized placebo-controlled trial 11 weeks	Garlic oil, 8.2mg/day Separate group, garlic powder, 1g/day	51 subjects	Healthy normo-lipidemic volunteers	None reported	No clinical relevance, as no adverse reactions reported
Effect of raw garlic *vs* commercial garlic supplements on Plasma Lipid concentrations Gardner, 2007	Parallel-design trial 6 months	4 arms: raw garlic, powdered garlic, aged garlic extract or placebo. Garlic product doses equivalent to an average-sized garlic clove were consumed 6 d/wk for 6 months.	192 adults	Adults with LDL-C concentrations of 130 to 190 mg/dL (3.36-4.91 mmol/L)	No serious adverse events. Rare reports of individual symptoms: rash, heartburn, and mouth ulcers (1 participant each). Bad body and breath odour were reported "often" or "almost always" by 28 participants (57%) in the raw garlic group and by 1 participant in the Kyolic group, but by no participants in the Garlicin or placebo groups. Flatulence attributed to study materials was reported "often" or "almost	No new safety concern. All the reported symptoms reflected in the monograph.

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
					always" by 3 participants in the raw garlic group, 4 participants each in the Garlicin and Kyolic groups, and 1 participant in the placebo group.	
Effect, tolerability and acceptability of aged garlic extract in patients with treated, but uncontrolled, hypertension Ried, 2010	Double-blind parallel - randomized placebo-controlled trial 12 weeks	Four capsules of aged garlic extract (960 mg containing 2.4 mg S-allyl-cysteine) daily for 12 weeks	50 patients	Hypertensive patients	Gastrointestinal side effects, belching, reflux, taste sensations	No new safety concern. All the reported symptoms reflected in the monograph.
Effect of aged garlic extract in hypertensive patients: dose response study Ried, 2013	Double-blind, randomized, placebo-controlled 12 weeks	Aged garlic extract: One, two or four capsules daily of aged garlic extract (240/480/960 mg containing 0.6/1.2/2.4 mg of S-allyl-cysteine) or placebo.	79 patients	Uncontrolled hypertension (SBP>140)	Gastrointestinal side effects in both groups. Garlic groups: Constipation, bloating, flatulence, reflux, garlic taste	No new safety concern. All the reported symptoms reflected in the monograph.

5.2. Patient exposure

Data obtained from more than 5000 patients, tested for safety during clinical trials, showed the adverse events listed under section 5.3

5.3. Adverse events, serious adverse events and deaths

The search in pharmacovigilance databases between January 2017 and October 2023 revealed 60 case reports. Among them, 40 cases were considered as severe and included mainly undesirable effects at gastrointestinal system and interaction with anti-coagulant drugs, ibuprofen (Hopf et al., 2008) or different preparations / herbals or food supplements (i.e. cod-liver-oil, echinacea).

One case of anaphylaxis after garlic ingestion has been also reported (Treudler et al., 2015).

These Undesirable effects and Interactions are already included in the current MO.

Gastro-intestinal irritation or allergic reactions may occur in rare cases. 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.

Bulbus Allii Sativi has been reported to evoke occasional allergic reactions such as contact dermatitis and asthmatic attacks after inhalation of the powdered drug (WHO, 1999). Those sensitive to garlic may also have a reaction to onion and/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 also reported (Rose et al., 1990).

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

A survey by Koch and Lawson (1995) showed that allergic reactions to garlic were reported in a total of 39 publications between 1938 and 1994 (Koch and Lawson 1995). Most of these cases involved an allergic contact dermatitis, sometimes severe (Eming *et al.,* 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 *et al.*1981; Papageogiou *et al.* 1983). Other reported side effects included bloating, headache, dizziness, and profuse sweating (Beck and Grunwald, 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, 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 *et al.,* 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, 1995)

The reported cases of post operative bleeding and spinal hematoma (German et al. 1995; Petry 1995; Burnham 1995; Rose et al.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, 2000; Kaye, 2000; Ang-Lee, 2001; Ciocon, 2004; Messina, 2006). However, the results obtained in two clinical trials (Scharbert *et al.* 2007; Beckert *et al.,* 2007) suggested that dietary garlic consumption does not affect platelet function.

Assessor comment:

In the absence of sufficient data, and for safety reasons, garlic consumption should be avoided 7 days before surgery, especially if high operative and postoperative bleeding risk.

Rapporteur's conclusion: Garlic has been an integral part of human diet for long time; it is taken for granted that garlic is safe in a wide range of doses. However, data from clinical 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.

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

No data available.

5.5.1. Use in children and adolescents

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, while it should have not been relevant, towards this indication.

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.

According to a review of data conducted by the University of Alberta (Shamseer *et al*, 2006), burns or contact dermatitis are the most noted adverse effects of garlic used topically even though the

cutaneous use of garlic is out of scope of this assessment and not included among proposed methods of administration. 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

5.5.2. Contraindications

Hypersensitivity to the active substance.

Patients under saquinavir/ritonavir therapy (see also section 5.5.4 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

An increased INR (International Normalized Ratio has been observed in 2 cases of patients under warfarin treatment who had used garlic preparations (Sunter, 1991).

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 monograph, 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).

In the study by Pisticelli (2001), 3-periods single sequence study was conducted in ten healthy volunteers receiving 10 doses of saquinavir 1200 mg three time per day with meal for 3 days and then they received garlic caplets twice daily from D5 to D24. Then, 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 garlic and saquinavir are metabolised by CYP450.

The study by Gallicano *et al*. (2003) 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 case-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.

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) (Markowitz et al., 2003). The study's duration was 14 days. The conclusions of this study were that garlic did not influence the pharmacokinetic of both components.

A clinical trial was conducted in 40 renal transplanted patients (Jabbari et al. 2005) receiving daily cyclosporine. They all received 1g of raw aged garlic by chewing or swallowing for a period of 2 months. Then, there was a period of one month of wash out and after that, the patients received garlic by the other route (chewing changed to swallowing and vice-versa) for another 2 months. This study did not demonstrate any change in cyclosporine's pharmacokinetic. The dose of cyclosporine was not known and not reported.

In a randomised, double-blind, placebo-controlled study by Macan *et al*., (2006), conducted in 66 patients (only 48 completed the study) to assess the effect of aged garlic extract (5ml twice daily for 12 weeks) on warfarin. There was no increased haemorrhage in any group and no modification of the INR in garlic arm was observed.

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

Docetaxel

One study was conducted in 10 women with metastatic breast cancer treated with docetaxel (30 mg/m²) for 3-4 weeks (Cox et al., 2006). Garlic was given twice daily at 600 mg in tablets from the third day after the first docetaxel dose and for a period of 12 days. There was not control group in this study. Garlic has referred that did not change docetaxel pharmacokinetic except for those patients with CYP3A5*1A allele.

Assessor's comment:

Several in vitro studies have assessed the effect of garlic on CYPP450 isoforms (Foster et al., 2011, Greenblatt et al., 2006, Ho et al. 2010, Zhou et al. 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, daily administration of garlic significantly increased hepatic CYP activity in vivo in rats (Dhamija et al., 2006; Dalvi 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.

The monograph should mention (and it has been mentioned) that garlic intake may reduce the effect of saquinavir/ritonavir.

In addition, considering the bleeding risk discussed in section 5.3, the monograph should advise (and has been mentioned too) that garlic supplements may increase bleeding times, and recommend using with caution garlic supplements with oral anticoagulation therapy and/or antiplatelet therapy.

5.5.5. Fertility, pregnancy and lactation

Pregnancy- Lactation

There are no objections to use garlic as food during pregnancy and lactation (because neither long-term nutritional experience nor any other important circumstances give reason for suspicion). Data from trials shown that major sulfur-containing volatiles from garlic are excreted in human milk, but its effect on the newborn have not been established (Mennella and Beauchamp, 1991).

Maternal garlic ingestion has a reputation for causing colic in breastfed infants. Two papers (Mennella and Beauchamp, 1991; 1993) tend to refute this claim. In one reference, 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 publication, 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 and Beauchamp 1993).

Adequate tests on reproductive toxicity and genotoxicity have not been performed.

Fertility studies in animals have shown effect on male fertility. Testicular toxicity (e.g. spermatogenesis impairment) was reported in rats treated for 30 days with crude garlic and in rats treated for 70 days with 50 mg of garlic powder. A decrease in testosterone occurs concomitantly; a NOAEL was not determined for the garlic powder. These effects on male rat fertility were observed at approximately twice the maximal human daily dose (see section 5.3 'Preclinical safety data' in the monograph).

Assessor's comment:

There are no data on use of garlic in pregnancy, out of common use as food, while there are no sufficient data to establish the safety of garlic preparations use during lactation. So, far the use of garlic in pregnancy and lactation is not recommended.

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.

The following tabulated list of adverse effects is included in Section 4.8 in the Monograph:

Frequencies not known.

Metabolism and nutrition disorders: decreased appetite.

Nervous system disorders: headache, dizziness.

Eye disorders: conjunctivitis.

Vascular disorders: haemorrhage.

Respiratory, thoracic and mediastinal disorders: rhinitis, bronchospasm.

Gastrointestinal disorders: breath odour, abdominal pain, abdominal distension, flatulence.

Skin and subcutaneous tissue disorders: hyperhidrosis, abnormal skin odour, contact dermatitis.

Description of selected adverse reactions:

Allergic reactions such as contact dermatitis, conjunctivitis, rhinitis, or bronchospasms, sometimes severe have been reported.

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. Garlic preparations should be used with caution in patients taking oral anticoagulation therapy and/or anti-platelet therapy because they may increase bleeding times.

Concomitant use with saquinavir/ritonavir is contraindicated because of the risks of decrease in plasma concentration, loss of virological response and possible resistance to one or more components of the antiretroviral regime (see also section 4.3 Contraindications and 4.5 Interactions)

On the basis of the information on its traditional use, garlic preparations prove not to be harmful in the specified conditions of use.

6. Overall conclusions

Well established use monograph

Several clinical studies have been conducted with *Allium sativum bulbus* preparations for the treatment of metabolic syndrome, cardiovascular disease risk factors (total cholesterol, triglycerides, and LDL-C levels, platelet activity or blood pressure) or atherosclerotic process, among others.

However, the HMPC was of the opinion that the placebo-controlled studies with *Allium sativum, bulbus* were not adequate to prove the efficacy of the preparations included in the EU monograph: the duration was not long enough, the number of patients included was low, the preparations assayed were not properly defined or were not included in the market overview and finally some studies were performed in non-EU countries.

Thus, the requirements for well-established use according to Article 10a of Directive 2001/83/EC are considered not fulfilled.

Traditional use monograph

It has been demonstrated that *Allium sativum bulbus* has been in traditional medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA, with an acceptable level of safety for 1) as an adjuvant for the prevention of atherosclerosis and 2) for the relief of the symptoms of common cold.

The requirements for traditional medicinal use according to Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC are considered fulfilled.

Herbal substance/preparation	**Indication**	**Therapeutic area for browse search**	**Posology and method of administration**	**Duration of use**
Powdered herbal substance	THMP used as an adjuvant for the prevention of atherosclerosis	Circulatory disorders	100 mg to 750 mg, 2 to 5 times daily Oral use	No restriction
	THMP for the relief of the symptoms of common cold	Cough and cold	600 mg, 3-4 times daily Oral use	1 week
Liquid extract from fresh bulb (DER 2-3:1), extraction solvent rapeseed oil, refined	THMP used as an adjuvant for the prevention of atherosclerosis	Circulatory disorders	110-220 mg, 4 times daily Oral use	No restriction
Dry extract (DER 5:1), extraction solvent ethanol 34% V/V	THMP for the relief of the symptoms of common cold	Cough and cold	100-200 mg, 1-2 times daily Oral use	1 week

For Indication 1) *THMP used as an adjuvant for the prevention of atherosclerosis*, the use in adolescents under 18 years of age has not been established due to the lack of data.

For Indication 2) *THMP for the relief of the symptoms of common cold,* the use in children under 12 years of age has not been established due to the lack of adequate data.

A European Union list entry is not supported due to lack of adequate data on genotoxicity.

Annex

List of references