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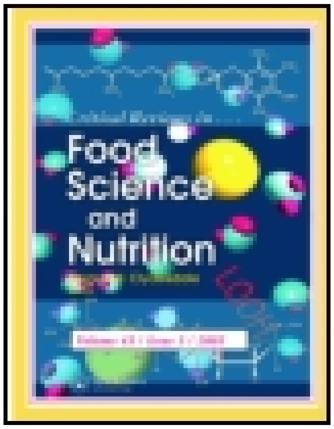

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Critical Reviews in Food Science and Nutrition

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Anti-cholelithogenic Potential of Dietary Spices and Their Bioactives

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Accepted author version posted online: 06 Jul 2015.

To cite this article: Krishnapura Srinivasan (2015): Anti-cholelithogenic Potential of Dietary Spices and Their Bioactives, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2014.1003783

To link to this article: http://dx.doi.org/10.1080/10408398.2014.1003783

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Gallstone prevention by hypocholesterolemic spices

Anti-cholelithogenic potential of dietary spices and their bioactives

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Abstract

Dietary hypocholesterolemic spices−curcumin (active compound of turmeric (*Curcuma longa*) and capsaicin (active compound of red pepper (*Capsicum annuum*), the active principles of spices – turmeric (*Curcuma longa*) and red pepper (*Capsicum annuum*), fenugreek (*Trigonella foenum-graecum*) seeds, garlic (*Allium sativum*), and onion (*Allium cepa*) are documented to have anti-cholelithogenic property in animal model. These spices prevent the induction of cholesterol gallstones by lithogenic high cholesterol diet and also regress the pre-established cholesterol gallstones, by virtue of their hypolipidemic potential. The antilithogenic influence of these spices is primarily attributable to their hypocholesterolemic effect. Increased cholesterol saturation index, cholesterol: phospholipid ratio and cholesterol: bile acid ratio in the bile caused by the lithogenic diet was countered by these spices. The anti-lithogenicity of these hypocholesterolemic spices was considered to be due also to their influence on biliary proteins which have pro-nucleating activity and anti-nucleating activity. Investigations on the involvement of biliary proteins in cholesterol crystal nucleation revealed that in an *in vitro* bile model, low molecular weight biliary proteins of the lithogenic diet fed animals have a pro-

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nucleating activity. On the contrary, low molecular weight biliary proteins of the animals fed hypocholesterolemic spices along with lithogenic diet showed a potent anti-nucleating activity.

Key words

Spices, Hypocholesterolemic effect, Anti-lithogenic effect, Biliary lipids, Cholesterol saturation index, Biliary proteins, Nucleating and anti-nucleating factors

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1. INTRODUCTION

Bile is a yellow viscous fluid, made in the liver and stored in the gallbladder that acts in the small intestine to facilitate digestion and absorption of dietary fat. The bile secreted by the liver flows through hepatic ducts to the gallbladder located under the liver, and stored until it is needed to digest dietary fat. After a meal, the gallbladder contracts and secretes bile through the common biliary-pancreatic duct in to the duodenum of the small intestine. Gallstone disease is a most common disorder affecting the body's biliary system, organs and ducts involved in the transport, store, and release of bile. The prevalence of cholesterol gallstone disease is very high in USA, Chile and Europe (Sweden, Germany, and Austria) (10-15%), followed by Asia (5-10 %) (Portincasa et al., 2006). The prevalence is very high in some ethnic groups: 60-70% in American Indians (female Pima Indians) and 35 % in Chilean Mapuche Indians. The overall prevalence rate is 7.9 % (men) and 16.6 % (women), with age >20 years (Cuevas et al., 2004).

Cholesterol gallstones (CGS) are abnormal mass of a solid mixture of cholesterol crystals, mucin, calcium bilirubinate and proteins. There are three types of gallstones depending on the major constituents they contain: cholesterol gallstones, pigment gallstones and mixed gallstones (**Table-1**). Majority of all gallstones are cholesterol stones, yellow-green stones made up of hardened cholesterol. Cholesterol stones are associated with bile that is supersaturated with cholesterol. Black pigment stones result from haemolysis and consist primarily of calcium bilirubinate, whereas brown pigment stones are associated with infections of the biliary tract (Portincasa et al.. 2006). Mixed gallstones consist of small amounts of calcium and bilirubin salts. Cholesterol gallstones which are yellow to gray in colour contain 50−90% cholesterol as major component, and are mainly formed by super saturation of bile with cholesterol (Portincasa

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et al.. 2006). Gallstones are usually asymptomatic in the beginning and symptoms become apparent once the stones reach a certain size (>8 mm). Gallstones can cause problems by lodging in bile ducts, hindering the flow of bile or digestive enzymes, and leading to abdominal pain, nausea, and inflammation (Grunhage and Lammert, 2006).

1.1. PATHOGENESIS

Cholesterol gallstones develop when bile contains too much cholesterol and not enough bile salts to hold them in solution. Besides a high concentration of cholesterol, two other factors seem to be important in causing gallstones. Incomplete and infrequent gallbladder contraction and emptying may cause the bile to become over-concentrated and contribute to gallstone formation (Festi et al., 1990). The presence of proteins in the liver and bile that either promote or inhibit cholesterol crystallization into gallstones may also be a factor in the pathogenesis of CGS (Holzbach et al., 1984). Thus, CGS forms when three conditions exist viz., (a) Bile is supersaturated with cholesterol, (b) The cholesterol in bile is rapidly transformed into crystals, and (c) There is a decrease in gallbladder contractions (Portincasa et al.. 2006). CGS occurs when the cholesterol concentration exceeds the bile's solubilising capacity. Thus, part of cholesterol precipitates in the form of micro crystals; these micro crystals aggregate to form microscopic structures. Alternatively, reduced contraction and reduced emptying of gallbladder that allows the bile to reside in the gallbladder for longer period enables to form cholesterol particles and further crystal growth (Festi et al., 1990). In supersaturated bile, phospholipids solubilise cholesterol into vesicles. Monohydrate crystals can precipitate from these cholesterol-enriched vesicles, and become entrapped in gallbladder mucin gel together with bilirubinate, and

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ultimately agglomerate into a macroscopic gallstone (Wang et al., 2009). Additionally, if pronucleating factors over-balance the anti-nucleating factors there is a high incidence of CGS.

1.2. RISK FACTORS

Obesity, aging, estrogen treatment, pregnancy and diabetes are associated with a higher risk of CGS (Grunhage and Lammert, 2006). Obesity is a significant risk factor for CGS, particularly for women with a body mass index of >30 (Cuevas et al. 2004). One explanation for the link between obesity and gallstones is that the overweight condition tends to upset the balance between cholesterol and other components of bile, either by reducing the other components relative to cholesterol or by causing excessive secretion of cholesterol into the bile. Obesity acts primarily by increasing the cholesterol synthesis, biliary cholesterol secretion and cholesterol super saturation. Obesity also slows down the emptying of the gallbladder. CGS formation is a most frequent and significant complication of rapid weight loss (Bellows et al. 2005; Shaffer, 2006). Cholesterol from fatty tissue are activated and secreted into the bile, leading to both cholesterol supersaturation and diminished gallbladder contractions. Prolonged fasting also decreases gallbladder movement and causes bile to over-concentrate cholesterol, leading to gallstones. During fasting, metabolism of the body fat secretes extra cholesterol in to bile, which also favors CGS formation. People with diabetes often have high levels of triglycerides in their blood, and these fatty acids tend to increase the risk of CGS (Biddinger et al., 2008).

The incidence of CGS increases with age, since there is a disordered hepatic sterol and bile acid metabolism as one ages resulting in the secretion of bile supersaturated with cholesterol. CGS is most common in women than men (Acalovschi, 2001). Excess estrogen from pregnancy, hormone replacement therapy and birth control pills appears to increase cholesterol levels in bile

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and decrease gallbladder movement, both these factors leading to CGS formation. Recent studies have suggested the involvement of regulatory genes of lipid metabolism in biliary lipid secretion and hence in CGS formation (Kosters et al., 2003). Thus, it is possible to hypothesize that diet modulation of these regulatory genes leading to an increase in biliary cholesterol secretion and/ or a decrease in the ratio bile salt/cholesterol in bile could favor cholesterol gallstone formation.

1.3. DIET AS A RISK FACTOR FOR CHOLESTEROL GALLSTONE DISEASE

A number of dietary factors have been involved in the pathogenesis of cholelithiasis (Portincasa et al., 2006). Low-fibre, high-cholesterol diets, and diets high in starchy foods have been suggested to contribute to CGS formation. Other nutritional factors that may increase risk of gallstones include rapid weight loss, constipation, and eating fewer meals per day. Considerable research has gone into examining the influence of dietary components like proteins, carbohydrates, fibre, fat, particularly cholesterol, and excess calorie intake on the pathogenesis of cholesterol gallstones (Cuevas et al., 2004; Mendez-Sanchez et al., 2007; Gaby, 2009). Consumption of simple sugars and saturated fat has been mostly associated to a higher risk, while fibre intake consistently reduces the risk (Portincasa, 2006).

Several experimental animal models have been employed for understanding the pathogenesis and possible approach to prevention and cure of gallstones. There is a risk of CGS associated with consumption of high amount of lipids, mainly saturated fatty acids. Animal studies have shown that monounsaturated fatty acids may decrease the risk of gallstone formation. Animals on diets containing polyunsaturated fat like fish oil have exhibited lower incidence of gallstone compared to those diets with saturated fat (Scobey et al., 1991). The mechanism involves changes in cholesterol metabolism that could lead to reduced biliary cholesterol saturation, combined with

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reduced biliary protein concentration and also prolonged nucleation time. Diets supplemented with cholesterol have been shown to produce lithogenic bile and gallstones in experimental animals. Thus, it has been hypothesized that a persistent high cholesterol intake may predispose to CGS, although human studies have yielded contradictory results. Consumption of high amounts of refined sugars generally increases the risk of CGS. This is attributed to a high synthesis of cholesterol in the liver secondary to an increase in insulin secretion. It has been shown that experimental animals on casein containing diet are more prone to CGS formation than those on soya protein (Ozben, 1989).

It is very well known that dietary fibre is beneficial in preventing CGS formation (Judd, 1985; Ebihara and Kiriyama, 1985). A large number of epidemiological studies have shown that insoluble fiber intake is inversely associated to gallbladder disease. Fibre protects gallstone formation by speeding intestinal transit time, reducing the generation of secondary bile acids such as deoxycholate and also inhibits CGS formation by reducing biliary cholesterol saturation. The anti-cholelithogenic potential of dietary tender cluster beans, a vegetable rich in soluble dietary fibre in experimental mice has been reported recently (Raghavendra and Srinivasan, 2014a). Dietary cluster bean significantly reduced the cholesterol gallstone incidence wherein biliary cholesterol was markedly reduced and hence the cholesterol saturation index. Dietary tender cluster beans exerted anti-cholelithogenic influence by decreasing the cholesterol hyper-secretion into bile and hence cholesterol saturation index, thus decreasing the formation of lithogenic bile in experimental mice.

Formation of cholesterol gallstones in gallbladder is controlled by procrystallizing and anticrystallizing factors present in bile in addition to super saturation of cholesterol. An animal

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study was conducted to evaluate the influence of soluble fibre-rich tender cluster beans on the compositional changes in the bile, particularly the effect on glycoproteins, low molecular weight (LMW) and high molecular weight (HMW) proteins, cholesterol nucleation time, and cholesterol crystal growth in rat hepatic bile and to assess the expression of CYP7A and CYP27 which are the major rate limiting genes in bile acid synthesis (Raghavendra and Srinivasan, 2015a). Incorporation of tender cluster beans (CB) into HCD decreased the cholesterol saturation index in bile by modulating cholesterol homeostasis, increased the bile flow rate and increased total proteins, particularly glycoproteins. Dietary CB prolonged the cholesterol nucleation time in bile. Electrophoretic profile of biliary proteins showed the presence of high concentration of 27 kDa protein which might be responsible for the prolongation of cholesterol nucleation time in the CB fed groups. Proteins of about 20 kDa and 18 kDa were expressed more in CB treated animals which prolonged the cholesterol nucleation time in rat bile, while the same was less expressed in HCD treatment. HMW and LMW proteins from CB fed animals were shown to reduce cholesterol crystal growth index (I_g) and crystal index (I_c) which were elevated in the presence of biliary proteins from HCD group. Cholesterol-7α-hydroxylase and cholesterol-27-hydroxylase mRNA expression was increased in CB treated animals by contributing to the major portion of the bile acid synthesis. These findings indicate that the beneficial anti-lithogenic effect of dietary CB which primarily is due to reduction in the cholesterol saturation index in bile, was additionally affected through a modulation of the nucleating and anti-nucleating proteins which in turn affect cholesterol crystallization.

It is reported that vitamin-C deficiency hinders the activity of cholesterol-7α-hydroxylase in liver, a regulatory enzyme in the conversion of cholesterol to bile acids, thus leading to cholesterol supersaturation in bile. It is proved that supplementation of Vitamin-C (2 g/day for 2 weeks) induces changes in bile composition and also prolongs the cholesterol nucleation time. Calcium has been hypothesized to protect against gallstones by binding secondary bile acids including deoxycholate in the small intestinal lumen, thus reducing the deoxycholate and cholesterol content of the bile (Cuevas et al., 2004). Some studies found an inverse association between dietary calcium and gallbladder disease.

2. BENEFICIAL INFLUENCE OF DIETARY SPICES ON CHOLESTEROL GALLSTONE

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Spices are being used as food adjuncts – as flavouring and colouring agents and as food preservatives for many centuries. The medicinal value of several spices in traditional systems of medicine has also been on record for a long time. Their health beneficial effects have been investigated more thoroughly in recent decades. Pioneering experimental research has documented several health beneficial attributes of spices *viz*., digestive stimulant action (Platel and Srinivasan, 2004), hypolipidemic effect (Srinivasan et al., 2004), anti-diabetic influence (Srinivasan, 2005), antioxidant potential (Srinivasan, 2014), anti-inflammatory property, anti-mutagenic and anti-carcinogenic potential (Srinivasan, 2005). In view of the several promising beneficial physiological effects spices are understood to exert, these food adjuncts are now considered as `nutraceuticals’ (Srinivasan, 2005).

2.1. DIETARY SPICES BENEFICIALLY MODULATE CHOLESTEROL HOMEOSTASIS

Among spices, garlic, onion, fenugreek, red pepper, and turmeric are well documented for their hypocholesterolemic and hypotriglyceridemic potential in various experimental animal models (Srinivasan et al., 2004). The cholesterol lowering potential of fenugreek in diabetic and normal subjects (Sharma, 1986; Sharma et al., 1990; Sharma and Raghuram, 1990; Sowmya and

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Rajyalakshmi, 1999) and of garlic in normal humans (Kleijnen et al., 1989; Grunwald, 1990; Auer et al., 1990; Vorberg, 1990; Brosche et al., 1990; Zimmermann and Zimmermann, 1990; Kiesewetter et al., 1990; Mader, 1990; Kiesewetter et al., 1991; Mansell and Reckless, 1991) or patients with coronary heart disease (Bordia, 1981) and of onion in normal subjects (Sainani et al., 1979; Bhushan et al., 1977; Sharma and Sharma, 1979; Jain et al., 1973) as well as in humans with induced lipemia (Jain, 1971; Sharma et al., 1975; Bordia et al., 1974; Jain and Andleigh, 1969; Jain and Vyas, 1977) has been demonstrated. While there are no reports on the hypocholesterolemic efficacy of the spices turmeric and red pepper or their bioactive compounds in humans, numerous studies are reported to possess significant hypocholesterolemic and hypo-triglyceridemic properties of the same in a variety of experimental situations in animals (Srinivasan et al., 2004). Capsaicin and curcumin − the bioactive compounds of red pepper and turmeric are documented to be efficacious at doses comparable to usual human intake (**Table-2**). Capsaicin and curcumin have been shown to be hypotriglyceridemic, thus preventing accumulation of fat in the liver under adverse situations by enhancing triglyceride transport out of the liver (Manjunatha and Srinivasan, 2007). Mechanism underlying the hypocholesterolemic and hypotriglyceridemic influence of these spices is fairly well understood. Health implications of the hypocholesterolemic effect of spices experimentally documented are cardio protection (Srinivasan, 2013), protection of the structural integrity of erythrocytes by restoration of membrane cholesterol/ phospholipid profile (Kempaiah and Srinivasan, 2005; Kempaiah and Srinivasan, 2006) and prevention of cholesterol gallstones by modulation of the cholesterol saturation index in bile (Srinivasan, 2013).

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It has been observed that in populations like the Indians, the incidence of cholesterol gallstone disease is much less than in others who do not consume spices so regularly. It is therefore very pertinent to examine if regular consumption of hypocholesterolemic spices will make less prone to the incidence of cholesterol gallstones. In recent years, some attention has been paid towards the possible role of spices or their active principles in influencing CGS formation. Spice principles – capsaicin and curcumin, and spices fenugreek, and onion have also been shown to be cholegoguic agents (Bhat et al., 1984; Bhat et al., 1985; Sambaiah and Srinivasan, 1991; Platel and Srinivasan, 2000; Platel et al. 2002); they enhance secretion of bile acids into bile. These hypocholesterolemic spices/ spice principles reduce blood and liver cholesterol by enhancing conversion of cholesterol to bile acids through activation of hepatic cholesterol-7α-hydroxylase, an enzyme having a regulatory role in cholesterol catabolism (Srinivasan and Sambaiah, 1991). One of the far-reaching implications of hypocholesterolemic influence is antilithogenic potential. In this context, all the five known hypocholesterolemic spices have been evaluated for their antilithogenic influence. The beneficial hypocholesterolemic property of the spices – turmeric and red pepper are attributable to the active principles (curcumin and capsaicin, respectively) present in them. It is believed that formation of cholesterol gallstone in the gallbladder is preceded by a supersaturation of bile with cholesterol (Admirand and Small, 1968). Hence, lowering of cholesterol concentration in the bile could prevent its supersaturation. A cholesterol lowering agent such as spices may therefore be able to reduce the incidence of CGS.

2.2. CURCUMIN AND CAPSAICIN − BIOACTIVE COMPOUNDS OF TURMERIC (CURCUMA LONGA) AND CHILLI PEPPER (CAPSICUM ANNUUM) IN THE PREVENTION OF CHOLESTEROL GALLSTONES **(Table-3)**

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The inhibitory effect of a curcuma mixture (*Temoe Lawak Singer*) on lithogenesis in rabbits has been reported by Beynen et al. (1987). Studies on experimental induction of cholesterol gallstones in mice and hamsters by feeding a lithogenic diet have revealed that the incidence of gallstones is 55-75% lower when the animals are maintained on 0.5% curcumin or 0.005% capsaicin containing diet (Hussain and Chandrasekhara, 1992; Hussain and Chandrasekhara, 1993). Biliary cholesterol concentration was also significantly reduced by spice principles feeding. The cholesterol saturation index which was 1.56 in animals fed lithogenic diet alone was considerably reduced to 0.54 and 0.35 in curcumin and capsaicin supplemented groups. The cholesterol: phospholipid ratio of bile was also reduced significantly in curcumin feeding.

Animal studies have also revealed significant regression of preformed cholesterol gallstones by these spice compounds in a 10 week mice feeding trial (Hussain and Chandrasekhara, 1994a). A 5-week feeding of curcumin and capsaicin resulted in 45 and 64% regression in preformed CGS, while the regression was still higher with 10 week feeding of these spice principles. The anti-lithogenicity of curcumin and capsaicin was considered to be due not merely to their ability to lower cholesterol saturation index, but also to their influence on biliary proteins (Hussain and Chandrasekhara, 1994b). This investigation on the involvement of biliary proteins in cholesterol crystal nucleation revealed that in an *in vitro* bile model, low molecular weight biliary proteins of the lithogenic diet fed animals showed a pro-nucleating activity. On the contrary, low molecular weight biliary proteins of the animals fed curcumin or capsaicin along with lithogenic diet showed a potent anti-nucleating activity. When capsaicin and curcumin were given together during experimental induction of cholesterol gallstone in mice, the combination although did not have an additive influence in reducing the incidence of CGS in mice, nevertheless was more

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beneficial in reducing the oxidative stress in lithogenic situation (Shubha et al., 2011). Reduction in CGS was accompanied by reduced biliary cholesterol and a marginal increase in phospholipid in these spice compounds fed groups. Increased cholesterol saturation index and cholesterol: phospholipid ratio in the bile caused by the lithogenic diet was countered by these two dietary spice compounds (Shubha et al., 2011).

2.3. ALLIUM SPICES (ALLIUM SATIVUM AND ALLIUM CEPA) IN THE PREVENTION OF CHOLESTEROL GALLSTONES (Table-4)

Dietary garlic (*Allium sativum*) and onion (*Allium cepa*), either raw or heat processed, included at 0.6 and 2.0% level, respectively, along with lithogenic high cholesterol diet for ten weeks reduced the CGS incidence in mice by 15-40%, the effect being maximum in heat processed onion group (Vidyashankar et al., 2009). Dietary garlic and onion markedly reduced biliary cholesterol, cholesterol: phospholipid ratio, and cholesterol saturation index. These *Allium* spices exerted antilithogenic influence by decreasing the cholesterol hyper secretion into bile and increasing the bile acid output thus decreasing the formation of lithogenic bile in experimental mice (Vidyashankar et al., 2009). Hepatic hydroxymethyl glutaryl-CoA reductase activity was lowered in lithogenic diet fed group, while dietary garlic or onion countered this alteration and also increased the activities of hepatic cholesterol-7α-hydroxylase and sterol-27-hydroxylase. Serum and liver cholesterol were decreased by feeding garlic or onion compared to lithogenic diet (Vidyashankar et al., 2009).

Dietary garlic and onion, either raw or heat-processed, also regressed pre-formed CGS in mice up to 50 ─ 60% while the regression in the basal control group was only 10%, thus suggesting that dietary garlic and onion effectively accelerate the regression of preformed CGS by

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promoting cholesterol desaturation in bile (Vidyashankar et al., 2010a). After inducing CGS in mice with a lithogenic diet for 10 weeks, they were maintained on basal diets containing 0.6% dehydrated garlic or 2% dehydrated onion for further 10 weeks. The anti-lithogenic potency of garlic was decreased by its heat processing, but not in the case of onion. Biliary cholesterol was significantly decreased in garlic and onion fed animals (Vidyashankar et al., 2010a). Biliary cholesterol saturation index and hydrophobicity index were significantly lowered by dietary garlic and onion. Serum and liver cholesterol levels were decreased by feeding these spices during post-CGS induction period (Vidyashankar et al., 2010a). Hepatic hydroxymethyl glutaryl-CoA reductase activity was increased after feeding garlic and onion, while activities of the cholesterol degrading enzymes− cholesterol-7α-hydroxylase and sterol 27-hydroxylase were increased in spice fed groups (Vidyashankar et al., 2010a). These results indicated that feeding garlic and onion effectively accelerate the regression of preformed CGS by promoting cholesterol desaturation in bile. This observation is significant in the context of evolving dietary intervention strategy to address regression of existing CGS and stopping the possible recurrence.

Formation of cholesterol gallstones in gallbladder is controlled by procrystallizing and anticrystallizing factors present in bile. The role of biliary proteins from rats fed lithogenic diet or garlic/ onion containing diet in the formation of cholesterol gallstones has been studied in model bile (Vidyashankar et al., 2010b). Cholesterol nucleation time of the bile from lithogenic diet group was prolonged when mixed with bile from garlic or onion fed groups. High molecular weight proteins of bile from garlic and onion fed groups delayed cholesterol crystal growth in model bile (Vidyashankar et al., 2010b). Low molecular weight (LMW) proteins from the bile of lithogenic diet group promoted cholesterol crystal growth in model bile, while LMW-protein

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fraction isolated from the bile of garlic and onion groups delayed the same (Vidyashankar et al., 2010b). Biliary LMW-protein fraction was subjected to affinity chromatography using Con-A and the lectin-bound and unbound fractions were studied for their influence on cholesterol nucleation time in model bile (Vidyashankar et al., 2010b). Major portion of biliary LMW-proteins in lithogenic diet group was bound to Con-A, and this protein fraction promoted cholesterol nucleation time and increased cholesterol crystal growth rate, whereas Con-A unbound fraction delayed the onset of cholesterol crystallization. Biliary protein from garlic/ onion group delayed the crystallization and interfered with pronucleating activity of Con-A bound protein fraction (Vidyashankar et al., 2010b). These data suggest that apart from beneficial modulation of biliary cholesterol saturation index, these two *Allium* spices also influence cholesterol nucleating and antinucleating protein factors that contribute to their antilithogenic potential.

A recent study evaluated the anti-cholelithogenic effect of a combination of tender cluster beans (*Cyamopsis tetragonoloba*) and garlic (*Allium sativum*) (Raghavendra and Srinivasan, 2014b). Lithogenesis was induced in mice by feeding a high (0.5%) cholesterol diet (HCD) for 10 weeks while tender cluster beans (CB) (10%) and garlic (1%) were included individually and in combination along with HCD. While all the three dietary interventions reduced cholesterol gallstones formation with attendant reduction in cholesterol in bile, serum, and liver, and countering of the elevated biliary cholesterol saturation index and cholesterol: phospholipid ratio in serum and liver, the combination of the two produced a higher effect (Raghavendra and Srinivasan, 2014b). Elevated lipid peroxides as a result of HCD were also reduced by these dietary interventions, the effect being greater with the combination (Raghavendra and

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Srinivasan, 2014b). Thus, the anti-cholelithogenic influence of dietary CB and garlic resulting from decreased cholesterol hyper-secretion into bile and hence the cholesterol saturation index and the related biochemical indices were higher with the combination of CB and garlic as compared to their individual effects.

A recent study evaluated the probable additive effect of a combination of dietary tender cluster beans and garlic on dissolution of pre-established cholesterol gallstones in mice ((Raghavendra and Srinivasan, 2015b)). Cholesterol gallstones were induced by feeding a high (0.5%) cholesterol diet for 10 weeks. Tender cluster beans (10% freeze-dried powder) and garlic (1% freeze-dried powder) were included individually and in combination in the basal diet of these groups of gallstone-induced animals for a subsequent period of 5 and 10 weeks. CB, garlic and their combination reduced the pre-established cholesterol gallstones by 61%, 50% and 72% respectively, in 10 weeks (Raghavendra and Srinivasan, 2015b). This was accompanied by a reduction in calculated cholesterol saturation index to 0.749, 0.903 and 0.648 from 1.86 in HCD group. This was supported by a beneficial modulation of altered ratios of cholesterol: bile acid and cholesterol: phospholipid ratios in the bile as a result of these dietary interventions (Raghavendra and Srinivasan, 2015b). Acceleration of the regression of preformed CGS in experimental animals lends further support to the health beneficial antilithogenic influence of dietary CB and garlic.

Another study also assessed the mRNA expression of CYP7A and CYP27 which are key enzymes that regulate the bile acid synthesis and play an important role in the elimination of cholesterol from body during the regression of cholesterol gallstones in mice in the same experimental design (Raghavendra and Srinivasan, 2015b). mRNA expression was quantified

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using RT-PCR and the results were analyzed using the formula $2^{-\Delta\Delta Ct}$ and fold change was calculated by considering control animal group value as a calibrator. mRNA expression of CYP7A in HCD group remained down-regulated at the end of 5 weeks as well as at the end of 10 weeks of CGS regression (Raghavendra and Srinivasan, 2015b). This trend on mRNA expression of CYP7A was reversed in 10% CB fed animals post-CGS induction. mRNA expression was even up-regulated at end of 10 weeks of this dietary regimen. Combination of cluster beans and garlic produced a higher up-regulation of mRNA expression of CYP7A at 10 weeks. A similar trend was observed in the mRNA expression of CYP27. These results on mRNA expression corroborate with the observed significant increase in the bile acid pool as a result of dietary interventions with cluster beans, garlic or their combination (Raghavendra and Srinivasan, 2015b).

2.4. FENUGREEK (TRIGONELLA FOENUM-GRAECUM) SEEDS IN THE PREVENTION OF CHOLESTEROL GALLSTONES (Table-5)

Dietary fenugreek seed has been evaluated for a beneficial role in the prevention and treatment of CGS in laboratory mice (Reddy and Srinivasan, 2009a). CGS was induced in groups of mice by maintaining on a lithogenic diet (0.5% cholesterol) for 10 weeks. Dietary fenugreek (5, 10 and 15%) significantly lowered the incidence of CGS in laboratory mice under lithogenic condition; the incidence was 63, 40 and 10% in 5, 10 and 15% fenugreek group respectively, as compared to 100% in lithogenic control, the effect being higher than those observed with other hypocholesterolemic spices ─ curcumin, capsaicin, garlic and onion (Reddy and Srinivasan, 2009a). The antilithogenic influence of fenugreek is attributable to its hypocholesterolemic effect and reduction in cholesterol saturation index in the bile. Parallel to the beneficial cholesterol

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lowering influence, biliary phospholipid content was also decreased by fenugreek treatment given along with the lithogenic diet. This decrease was of the order of 22-33%. Bile salts form a major part of bile solids, and the majority of the cholesterol is excreted from the body after being converted into bile acids in the liver and subsequently secreted into the bile. Although bile acids remained unaffected by dietary fenugreek under lithogenic conditions, cholesterol: bile acids ratio was diminished due to lowered biliary cholesterol content.

Fenugreek seeds also showed beneficial antilithogenic influence in terms of regression of pre-established cholesterol gallstones (Reddy and Srinivasan, 2009b). After the CGS induction by feeding a high cholesterol diet for a period of 10 weeks, groups of these animals were maintained for further 10 weeks on high cholesterol/ basal control diet/ 6% or 12% fenugreek powder diets. CGS was significantly lowered as a result of dietary fenugreek seeds, the extent of regression being over 60% when compared to 10% regression in basal control diet group (Reddy and Srinivasan, 2009b). The antilithogenic influence of dietary fenugreek was accompanied by significant reductions in cholesterol concentration in serum, liver and bile. Biliary cholesterol: phospholipid ratio, cholesterol: bile acid ratio and cholesterol saturation index were lowered much more than basal control group upon feeding fenugreek during post-CGS induction period (Reddy and Srinivasan, 2009b). This study has evidenced the potency of hypolipidemic fenugreek seeds in regressing the pre-established CGS and this beneficial antilithogenic influence is attributable to its primary influence on cholesterol levels. Another report has suggested the hepatoprotective and antioxidant potential of dietary fenugreek seeds under conditions of lithogenicity (Reddy and Srinivasan, 2011a). These findings are significant in the

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context of evolving a dietary strategy to address CGS, which could help in the prevention of incidence, regression of existing CGS and preventing possible recurrence.

An animal study has evaluated the effect of dietary fenugreek on the compositional changes in the bile, particularly effect on glycoproteins, low molecular weight and high molecular weight proteins, cholesterol nucleation time, and cholesterol crystal growth (Reddy and Srinivasan, 2011b). Incorporation of fenugreek into the lithogenic diet decreased the cholesterol content, total protein, glycoprotein, lipid peroxides and cholesterol saturation index in bile, and increased the bile flow rate. Incorporation of fenugreek into the lithogenic diet also prolonged the cholesterol nucleation time, reduced the vesicular form of cholesterol (65% decrease) which was accompanied with an increase in the smaller vesicular form (Reddy and Srinivasan, 2011b). Electrophoretic separation of biliary LMW proteins showed the presence of higher concentration of 28 kDa protein which might be responsible for the prolongation of cholesterol nucleation time in the fenugreek fed groups (Reddy and Srinivasan, 2011b). These findings indicate that the beneficial antilithogenic effect of fenugreek which is primarily attributable to a reduction in the cholesterol content of the bile is also complemented through a modulation of the nucleating and anti-nucleating proteins which in turn affect the cholesterol crystallization.

An animal study was conducted to evaluate the antilithogenic effect of a combination of dietary fenugreek seeds and onion (Reddy and Srinivasan, 2011c). Fenugreek seeds (12%) and onion (2%) was included individually and in combination in a high (0.5%) cholesterol diet for 10 weeks. Fenugreek, onion and their combination significantly reduced the incidence of cholesterol gallstones with attendant reduction in cholesterol content in serum, liver and bile. Cholesterol saturation index of bile was significantly reduced by the combination of fenugreek and onion

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(Reddy and Srinivasan, 2011c). Inflammation of gallbladder membrane produced by HCD was reduced by fenugreek, onion and their combination. The antilithogenic influence was highest with fenugreek alone and the presence of onion along with it did not further increase this health effect. There was also no additive effect of the two spices in the recovery of antioxidant molecules or in the antioxidant enzyme activities (Reddy and Srinivasan, 2011c).

3. SUMMARY AND CONCLUSION

Cholesterol gallstone disease has emerged as one of the complex disease involving liver, gall bladder and intestine. CGS is a complex interaction of genetic and environmental factors. New findings highlight gene transcription, protein function, and regulation of lipid metabolism to have an important role to play in CGS. Factors such as age, pregnancy, obesity, diabetes, rapid weight loss, prolonged fasting, sedentary life style, consumption of refined sugar and saturated fat have been mostly associated with higher risk for this disease. It is apparent that high energy intake and energy storage which are related to obesity represent an important risk factor for the formation of gallstones, presumably through hyperinsulinism. Fibre-rich food and moderate consumption of alcohol appear to reduce the risk of cholesterol gallstone formation. New knowledge related to the molecular genetic regulatory mechanisms of hepatic cholesterol metabolism and secretion into bile may lead to better understanding of the role of diet in CGS formation. Dietary curcumin (0.5%) of turmeric, capsaicin (0.015%) of chilli pepper, fenugreek seeds (5-15%), garlic (0.6% powder) and onion (2% powder) have been reported to cause a significant reduction in the formation of gallstones in mice/ hamsters maintained on a lithogenic high cholesterol diet. Further, these spices/ spice principles effected a marked regression of pre-established gallstones in mice. Increased cholesterol saturation index, cholesterol: phospholipid ratio and cholesterol:

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bile acid ratio in the bile caused by lithogenic diet was countered by these dietary spices/ spice compounds. The influence of a hypocholesterolemic agent on biliary excretion of cholesterol and bile acids is linked closely with its influence on cholesterol homeostasis. The anti-cholelithogenic influence of spices are attributable to the cholesterol-lowering effect of these in blood and liver (**Fig.1**), and their ability to lower cholesterol saturation index by altering the bile composition and also to their influence on biliary proteins (**Fig.2**).

There are no human studies so far supporting the benefit of cholesterol lowering spices in the prevention or regression of cholesterol gallstones. Hence, the observed evidences from animal studies need to be further substantiated in terms of examining for a similar beneficial potency of hypocholesterolemic spices in human situation. Such studies would enable the exploitation of the therapeutic potential of spices or their active principles in the prevention and treatment of cholesterol gallstone disease.

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ABBREVIATIONS

CB, Cluster beans; CGS, Cholesterol gallstones; CoA, Coenzyme A; HCD, High cholesterol diet; HMW, High molecular weight; kDa, Kilo Dalton; LMW, Low molecular weight; mRNA, Messenger ribonucleic acid; RT-PCR, Reverse transcription polymerase chain reaction;

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Table-1. Different classes of gallstones and their characteristics

Type of gallstone	Cholesterol gallstones	Pigment stones	
		Brown	**Black**
Prevalence	85-90%	10-15%	≤ 5%
Main constituent	50-90% Cholesterol	≈ 50% bilirubin	≥50% bilirubin
Colour	Yellow to gray	Brown	Dark brown-black
Etiology	Cholesterol supersaturation	Increased deconjugation of bilirubin	Increased biliary bilirubin load
Location of affliction	Gallbladder	Bile duct	Gallbladder

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Table-2. Dietary intake of common spices by Indian population

Spice /Bioactive compound	**Intake/day g / adult***
Fenugreek seeds (*Trigonella foenum-graecum*)	0.3 - 0.6
Garlic (*Allium sativum*)	0 - 20
Onion (*Allium cepa*)	0 - 500
Red pepper (*Capsicum annuum*)	2.4 - 4.1
Capsaicin[a]	0.007 - 0.012
Turmeric (*Curcuma longa*)	0.007 - 0.012
Curcumin[b]	0.004 - 0.1

* Adult body wt. average 60 kg; a: 300 mg% in red pepper; b: 2% in turmeric

(Source: Thimmayamma et al., 1983)

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Table-3. Anti-cholelithogenic effect of hypocholesterolemic spices− turmeric (*Curcuma longa*)/ curcumin and chilli pepper (*Capsicum annuum*)/ capsaicin in experimental animals

Animal Model	Effect demonstrated	Researcher
Rats fed normal diet	Feeding of 0.2 and 0.4% curcumin increased biliary total bile acids	Bhat et al. 1984
Rats fed normal diet	Feeding of 7.5 and 15 mg% capsaicin increased biliary total bile acids	Bhat et al. 1984
Rats fed normal diet	3 specific spice mixes which contain red pepper and turmeric among others brought about a pronounced stimulation of bile flow and bile acid secretion	Platel et al. 2002
Dogs	Increased secretion of biliary cholesterol and bile acids by sodium curcuminate (25 mg/kg) administered by gavage	Ramaprasad & Sirsi, 1975
Rabbits	The inhibitory effect of a curcuma mixture (*Temoe Lawak Singer*) on lithogenesis. Curcuma mixture partly counteracted the increased lithogenic index of bile when fed with cholesterol.	Beynen et al. 1987
Lithogenic diet fed Mice	Dietary curcumin (0.5%)/ capsaicin (0.015%) reduced the formation of gallstones; The anti-lithogenic influence was attributable to lowering of cholesterol saturation index	Hussain & Chandra-sekhara, 1992
Lithogenic diet fed Hamsters	Dietary curcumin (0.5%)/ capsaicin (0.015%) reduced the formation of gallstones; The anti-lithogenic influence was attributable to lowering of cholesterol saturation index	Hussain & Chandra-sekhara 1993

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Lithogenic diet fed Mice	Dietary curcumin (0.5%) and capsaicin (0.015%) effected a marked regression of pre-established gallstones in mice	Hussain & Chandra-sekhara, 1994a
Lithogenic diet fed rats & Model bile system	The anti-lithogenicity of curcumin and capsaicin was also considered to be due to their influence on biliary proteins	Hussain & Chandra-sekhara, 1994b
Lithogenic diet fed Mice	Capsaicin and curcumin given together was more beneficial in reducing the oxidative stress in lithogenic situation	Shubha et al. 2011

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Table-4. Anti-cholelithogenic effect of hypocholesterolemic Allium spices– garlic (*Allium sativum*) and onion (*Allium cepa*) in experimental animals

Animal Model	Effect demonstrated	Researcher
Rats fed normal diet	Feeding 3% of onion powder increased biliary total bile acids; Dietary onion also increased cholesterol excretion in the bile	Sambaiah & Srinivasan, 1991
Rats fed normal diet	Dietary garlic powder decreased biliary cholesterol secretion.	Platel & Srinivasan, 2000
Lithogenic diet fed mice	Dietary garlic and onion (0.6 and 2.0%, respectively) reduced the CGS incidence by 15–40%. The Allium spices exerted antilithogenic influence by decreasing the cholesterol hyper secretion into bile and reducing cholesterol saturation index	Vidyashankar et al. 2009
Lithogenic diet fed mice	Dietary garlic and onion also regressed pre-formed CGS in mice up to 50–60%, while the regression in control group was only 10%	Vidyashankar et al. 2010a

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Lithogenic diet fed rats	*Allium* spices also influence cholesterol nucleating and antinucleating proteins that contribute to their antilithogenic potential.	Vidyashankar et al. 2010b
Lithogenic diet fed mice	Anti-cholelithogenic effect of a combination of tender cluster and garlic was higher than their individual effects.	Raghavendra & Srinivasan, 2014b
Lithogenic diet fed mice	Regression of preformed CGS in experimental animals was accelerated by dietary tender cluster beans and garlic, the beneficial effect being higher in combination.	Raghavendra & Srinivasan, 2014c
Lithogenic diet fed mice	mRNA expression corroborated with the observed significant increase in the bile acid pool as a result of dietary interventions with cluster beans, garlic or their combination.	Raghavendra & Srinivasan, 2014c

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Table-5. Anti-cholelithogenic effect of dietary fenugreek seeds (*Trigonella foenum-graecum*) in experimental animals

Animal Model	**Effect demonstrated**	**Researcher**
Rats fed normal diet	Feeding 0.5 and 2% of fenugreek increased biliary total bile acids	Bhat et al. 1985
Lithogenic diet fed mice	Dietary fenugreek (5, 10, and 15%) significantly lowered the incidence of CGS. The antilithogenic effect is attri-butable to hypocholesterolemic influence and reduction in cholesterol saturation index in the bile	Reddy & Srinivasan, 2009a
Lithogenic diet fed mice	Dietary fenugreek also regressed pre-established cholesterol gall-stones, the extent of regression being over 60% when compared to 10% in control diet group	Reddy & Srinivasan, 2009b
Lithogenic diet fed rats	The beneficial anti-lithogenic effect of fenugreek is also complemented through a modulation of the nucleating and anti-nucleating proteins which affect	Reddy & Srinivasan, 2010

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	cholesterol crystallization.	
Lithogenic diet fed mice	Hepatoprotective and antioxidant potential of fenugreek seeds were evident under lithogenic conditions.	Reddy & Srinivasan, 20011a
Lithogenic diet fed mice	Feeding of onion along with fenugreek did not further increase anti-lithogenic influence. There was also no additive effect of the two spices in the recovery of antioxidant status.	Reddy & Srinivasan, 20011b

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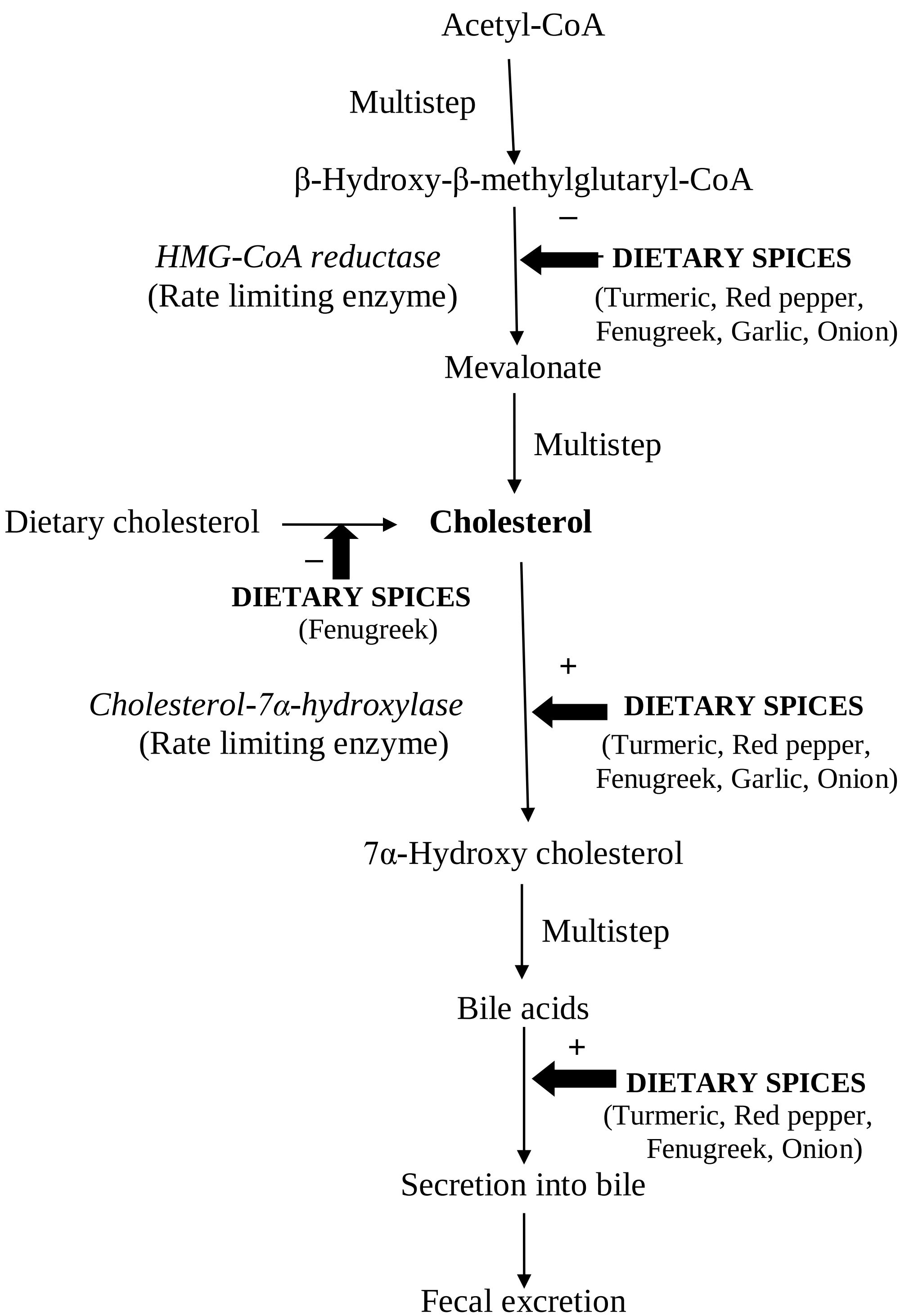

Fig.1. Beneficial modulation of cholesterol homeostasis by dietary spices by: (a) Intervention of dietary uptake, (b) Inhibition of *de novo* synthesis in liver, and (c) Stimulation of conversion to bile acids in liver (d) Higher secretion of bile acids into bile

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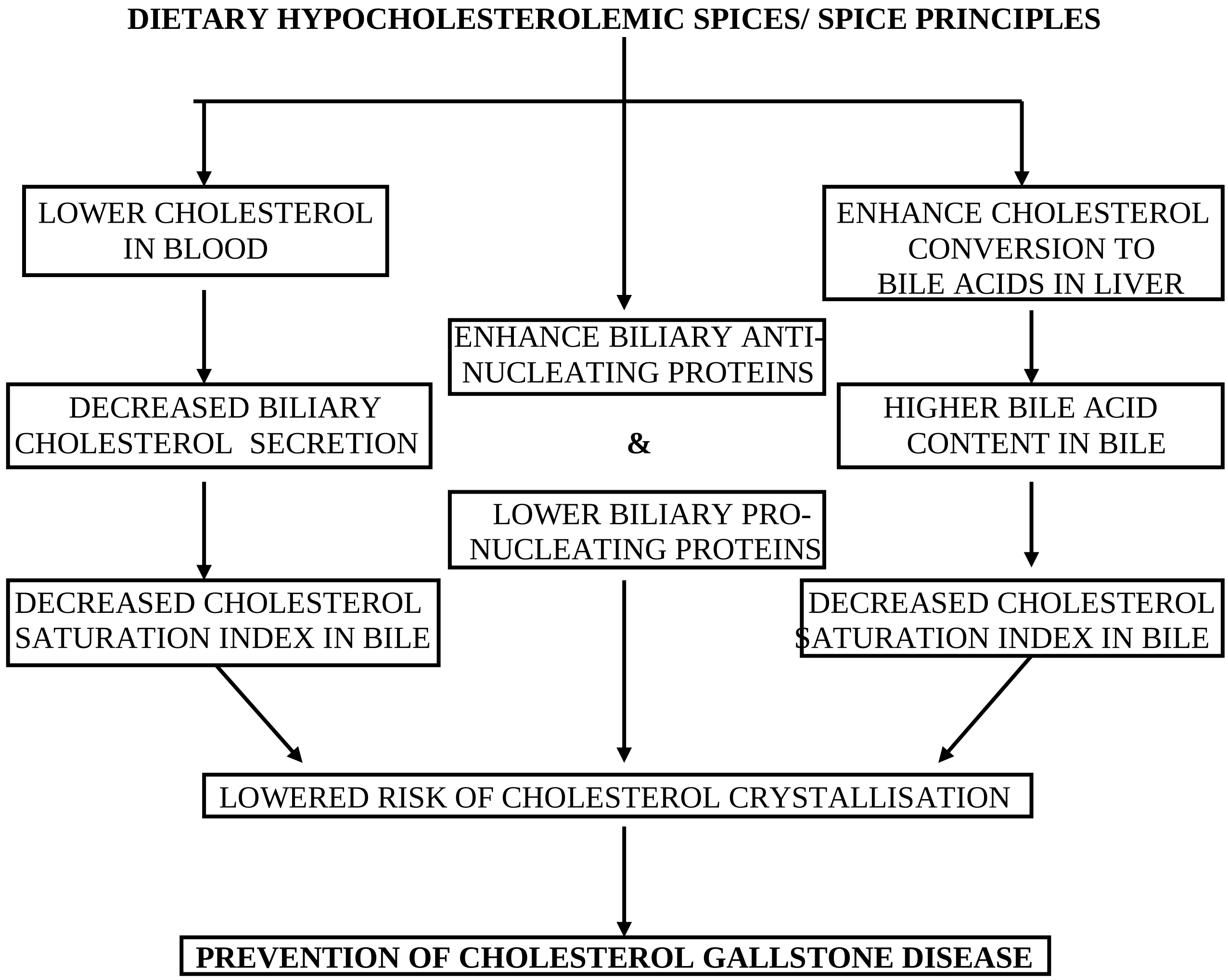

Fig. 2. Mechanism of Anti-cholelithogenic effect of dietary hypocholesterolemic spices

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