

# *Raphanus sativus* L. var *niger* as a source of Phytochemicals for the Prevention of Cholesterol Gallstones

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***Raphanus sativus* L. var *niger* (black radish) is a plant of the cruciferous family with important ethnobotanical uses for the treatment of gallstones in Mexican traditional medicine. It has been established that the juice of black radish decreases cholesterol levels in plasma and dissolves gallstones in mice. Glucosinolates, the main secondary metabolites of black radish, can hydrolyze into its respective isothiocyanates and have already demonstrated antioxidant properties as well as their ability to diminish hepatic cholesterol levels; such therapeutic effects can prevent the formation of cholesterol gallstones. This disease is considered a current problem of public health. In the present review, we analyze and discuss the therapeutic effects of the main glucosinolates of black radish, as well as the effects that this plant has on cholesterol gallstones disease. Copyright © 2013 John Wiley & Sons, Ltd.**

*Keywords:* *Raphanus sativus* L. var *niger*; gallstones; glucosinolates; cholesterol.

## INTRODUCTION

Cholesterol gallstones are a highly prevalent affection in Western countries such as the United States; in Latin America, it is prevalent in countries such as Chile, Mexico and Argentina (Portincasa *et al.*, 2006). As this disease produces a high number of medical consultations and hospital admissions, it is thought to be a public health problem; its pharmacological treatment is very limited (Di Ciaula *et al.*, 2010), and the invasive prophylactic therapy for symptomatic gallstones is cholecystectomy, or the removal of the gallbladder (Portincasa *et al.*, 2012). Because of this, research focuses on the search for preventive treatments; the possible preventive effects of numerous proteins that participate in the transport and metabolism of biliary lipids have been investigated, such as ATP or ABC-binding cassette: ABCB11, ABCB4, ABCG5 and ABCG8 (Castro-Torres, 2012; Stokes and Lammert, 2011). Treatments based on traditional medicine are highly relevant, and researching them is a complex matter. In Mexican traditional medicine, the black radish has been used since ancient times for treating gallstones (Castro-Torres and Naranjo-Rodríguez, 2012), stimulating the biliary function and to treat some digestive problems caused by the high intake of fats (Lugasi *et al.*, 2005). It is now known that black radish juice can be used for treating gallstones in mice fed with a lithogenic diet (rich in cholesterol and cholic acid) and that it has the property of diminishing serum triglycerides and cholesterol

levels, important effects that not only serve for treating the disease, but also to prevent it (Castro-Torres *et al.*, 2012). The secondary metabolites of the black radish are mainly glucosinolates, of which glucoraphasatin represents more than 65% of them (Hanlon *et al.*, 2007); this component and its hydrolysis product, called raphasatin, have demonstrated to possess antioxidant properties in different biological models and that these effects can prevent the appearance of oxygen reactive species, which can appear in conditions that are also suitable for the appearance of gallstones, due to the high concentration of hepatic cholesterol. Oxygen reactive species damage the gallbladder epithelium and, due to the constant secretion of biliary cholesterol, produce a pathophysiological imbalance which ends with the formation of a gallstone (Koppiseti *et al.*, 2008); therefore, glucoraphasatin and raphasatin could be two natural agents that prevent both the formation of oxygen reactive species and cholesterol gallstones. Another glucosinolate present in black radish is glucoraphanin, which yields sulforaphane as a product of its immediate hydrolysis; in addition to the antioxidant properties of these metabolites, they also diminish hepatic cholesterol levels by inhibiting the expression of enzymes and transcription factors associated with cholesterol metabolism (Rodríguez-Cantú *et al.*, 2011). Black radish contains important metabolites for the prevention of gallstones; in this review, we discuss the reports that support this therapeutic mechanism.

## *Raphanus sativus* L. var *niger*

This plant, popularly known as black radish, belongs to the cruciferous family, which includes cabbage, cauliflower, red

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radishes, spinach and broccoli (Sipos *et al.*, 2002). The origin of black radish is not known exactly; nevertheless, in many Asian and African regions, it is used for treating different problems associated with the gastrointestinal, hepatic and biliary systems (Vargas *et al.*, 1999). Its effects have not been scientifically demonstrated; they are only known from ethnobotanical information. In Mexico, the plant is used to treat pigmented and cholesterol gallstones (Castro-Torres *et al.*, 2012). We know that treatment is a different concept from prevention; in the search for curative and preventive treatments for cholesterol gallstones, the essential thing is to diminish the concentration of intestinal, hepatic and biliary cholesterol, and also, although less importantly, of plasmatic cholesterol. Black radish is capable of diminishing serum lipids in C57BL/6 mice, an important species that develops gallstones (Castro-Torres and Naranjo-Rodríguez, 2012); the decrease of serological cholesterol can be a factor associated with the development of gallstones, but it does not necessarily indicate that there is a constant biliary lipid secretion; nevertheless, black radish can dissolve cholesterol gallstones in mice (Castro-Torres *et al.*, 2012), which demonstrates the strong effect it has on cholesterol that is already crystallized and inside a matrix of glycoproteins. Moreover, if it can disintegrate gallstones, it is probable that it can diminish biliary cholesterol concentration; these factors are indeed essential for avoiding the formation of lithiasis. No toxicity related to the different therapeutic effects of black radish has been reported, but, with respect to cholesterol gallstones treatment, we have reported that the  $DL_{50}$  of black radish juice is over 5000 mg/kg (Castro-Torres *et al.*, 2012), which allow us to think that the natural product is not toxic, that it is effective and that it is not necessary to look for a lethal dose at higher values than the one we analyzed because therapeutic effects produced with minimal doses are preferred. In Mexico, there are also ethnobotanical uses of the plant for the treatment of pigment gallstones, which have a pathophysiology different from that of cholesterol gallstones; calcium salts of bilirubin abound in the first ones, crystallized cholesterol in the second ones (van Erpecum, 2011); it would be important to start new studies in order to analyze the effect of black radish in pigmented gallstones, which do not have pharmacological treatment, only surgical.

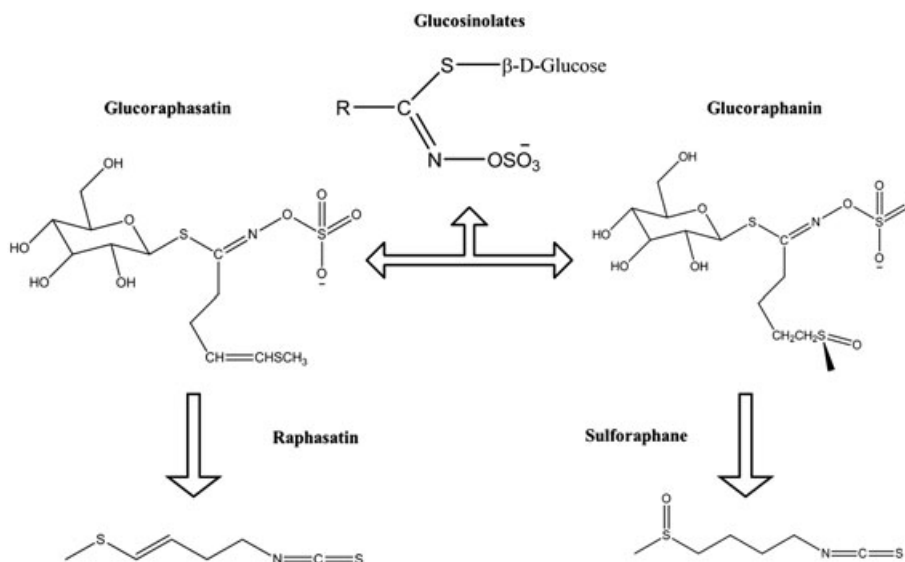
### Cholesterol gallstones

Cholesterol gallstones are one of the most frequent gastroenterological affections, representing a gallbladder disease (Portincasa *et al.*, 2006); its prevalence in Western countries is 14–16%, caused by obesity and a high-fat diet. In Latin America, Chile and Mexico are the countries with the highest prevalence of cholesterol gallstones; in these regions, the genetic background is a determining factor (Stinton *et al.*, 2010). There are numerous risk factors for the development of cholesterol gallstones: female sex, obesity, sedentary lifestyle, high-fat diet, advanced age, hypercholesterolemia, among others (Stokes *et al.*, 2011). The pathophysiology of gallstones is complex and polygenic; nevertheless, there are two processes related to cholesterol transport and use that are highly relevant for preventing the formation of gallstones: biliary secretion and intestinal absorption (Portincasa and Wang, 2012). Nowadays, there is a

medicine, called ezetimibe, which inhibits the expression of the Niemann-Pick type C1 type 1 (NPC1L1) protein and thereby diminishing the rate of intestinal absorption of cholesterol (de Bari *et al.*, 2012). Ezetimibe inhibits the formation of cholesterol gallstones in experimental models (Wang *et al.*, 2008; Zúñiga *et al.*, 2008); regrettably, there are no specific clinical studies that can evaluate the effect of NPC1L1 expression in the prevention of gallstones. The pharmacological treatment has its limitations; the medications used as prophylactics are: ursodeoxycholic acid, ezetimibe and statins (Di Ciaula *et al.*, 2010); it is important to mention that when gallstones are already formed, these therapeutic agents present a low capacity to dissolve them, ursodeoxycholic acid being the most used medicine for it, but, in order to work, gallstones should be strictly of cholesterol, and they must be within a gallbladder with adequate motility. The search for a therapeutic target is focused on biliary secretion and the intestinal absorption of cholesterol, but it is very important to consider also the production of reactive oxygen species, which are produced by cholesterol loading and that cause secretion rates to exceed normal levels. Considering these species to be target molecules and knowing that they can be produced in both hepatocytes and in the vesicular epithelium, the glucosinolates and their degradation products (with antioxidant capacity) that are present in black radish might impede the formation of gallstones in the gallbladder and at the same time diminish biliary cholesterol secretion by reducing hepatic cholesterol.

### Glucosinolates

Glucosinolates are biologically active secondary metabolites that play an interspecific and intraspecific role in the Brassicaceae family of plants (Sønderby *et al.*, 2010). Chemically, they are anionic glycosides (Fig. 1), and their content changes depending on the species, the part of the plant and climatic conditions (Agerbirk and Olsen, 2012). Glucosinolates possess structural diversity, reflecting the fact that their biogenetic precursors are tyrosine, phenylalanine and tryptophan (Sønderby *et al.*, 2010). These secondary metabolites are inside vacuoles; myrosinase is the enzyme responsible for hydrolyzing them into different degradation products, such as nitriles, isothiocyanates and thiocyanates (Peñas *et al.*, 2011). The strong flavor that glucosinolates and their derivatives give to cruciferous plants is associated with a defense mechanism against herbivorous organisms (Mithöfer and Boland, 2012). Among the most studied glucosinolates, there are two compounds, glucoraphasatin and glucoraphanin, found mainly in radishes and broccoli. Glucoraphasatin has demonstrated antioxidant effects in cancer models *in situ*, as has its degradation product, an isothiocyanate called raphasatin (Hanlon *et al.*, 2007). Glucoraphanin and its immediate product, sulforaphane, also have antioxidant properties and can diminish the levels of plasmatic cholesterol (Rodríguez-Cantú *et al.*, 2011; Boddupalli *et al.*, 2012). The red radish (*Raphanus sativus* L.) is a cruciferous rich in isothiocyanates; its administration to C57BL/6J mice under a hypercholesterolemic diet can reduce serum triglycerides and cholesterol levels (Balasinska *et al.*, 2005). The metabolism of cholesterol and triglycerides is strongly associated with gallstones



**Figure 1.** Major glucosinolates and their degradation products.

formation (Smelt, 2010), and C57BL/6J mice are susceptible models for their development. The ethnobotanical uses of radishes and broccoli include diminishing cholesterol levels and as antioxidants in, but there is no current wide scientific support for this, and we have a long way ahead before determining the active secondary metabolites and their action mechanism.

### Glucoraphasatin and antioxidant effects

Glucoraphasatin and its hydrolysis product, raphasatin, are known for their antioxidant properties, which have been related to cancer prevention (Fahey *et al.*, 2012); nevertheless, these properties have not been extensively explored with respect to other pathologies. The absence of antirust nutrients and an imbalance in glutathione homeostasis, the main antioxidant molecule of the human organism, are typical in cholesterol gallstones disease (Worthington *et al.*, 2004). Glucoraphasatin is the main glucosinolate of black radish, identified in high concentrations in aqueous extracts (approximately 30  $\mu\text{mol}$  of glucoraphasatin for each gram of dry vegetable material). In animal models, the levels of glutathione peroxidase in erythrocytes are increased by the black radish juice; the possible active metabolite might be glucoraphasatin (Lugasi *et al.*, 2005). In Hungary, a study was carried out to evaluate the juice extracted from the root of black radish; they used Wistar male rats fed with a hyperlipidaemic diet (cholesterol 2%, colic acid 0.5% and fat 20%). Later, these rats were treated with black radish juice for 9 days, administered to them on demand and diluted 10 times in water. The rats that received only a hyperlipidaemic diet had high serological concentrations of primary and secondary lipid peroxidation products, while in the animals treated with black radish juice, these peroxidation products diminished significantly. Lipid-rich diets also alter the blood antioxidant system; therefore, in rats treated with a high fat diet, the concentration of glutathione peroxidase diminished significantly, as opposed to what happened to rats treated with black radish juice, which showed a higher level of this enzyme (Lugasi *et al.*, 2005). This study is crucial, associated with cholesterol gallstones prevention.

The diet administered to Wistar rats works as a lithogenic diet in mice; the diet produces cholesterol gallstones in mice, while rats cannot produce them because they lack a gallbladder. The treatment with black radish juice had significant antioxidant effects in rats, the same that can be expected in mice, which have the ability to prevent the formation of gallstones. Black radish, in contrast to other cruciferous plants, is used in traditional medicine to prevent and to dissolve cholesterol and pigmented gallstones, aspects that reinforce the scientifically demonstrated therapeutic effects and that are associated to biliary lithiasis prevention. After assessing the antioxidant properties of black radish with *in vitro* models, the investigations continued with *in situ* models.

Antioxidant enzymes can prevent the formation of reactive oxygen species, which generate problems with the gallbladder's motility; this is one of the main factors that predispose to the development of gallstones. It is feasible to consider the study of metabolites that are capable of activating the expression of antioxidant enzymes in order to avoid gallstones formation; for example, raphasatin (isolated from black radish) is a powerful enzymatic detoxification inductor. In (HepG2) hepatic cell lines and in a concentration of 10  $\mu\text{M}$ , this metabolite induces messenger RNA expression of important phase II detoxification enzymes: quinone reductase, hemoxygenase and thioredoxin reductase (Hanlon *et al.*, 2007). A study carried out in the United States demonstrated that the aerial parts of black radish (stems and leaves), in contrast to the root, more effectively induce the expression of hepatic detoxification phase I and II antioxidant enzymes (Hanlon *et al.*, 2009). This analysis, carried out in cell lines, demonstrates a significant antioxidant effect. In biliary lithiasis conditions, reactive oxygen species can act at the hepatic and biliary level; in the gallbladder, they produce cellular infiltration, edema, granular hyperplasia and hypersecretion of mucin, which facilitates the formation of biliary sludge and accelerates the development of gallstones (Koppiseti *et al.*, 2008). Also, these species facilitate cholesterol crystallization, while enzymes with antioxidant capacity can prevent many pathophysiological disorders related to gallstones.

## Glucoraphanin and cholesterol-lowering effects

Glucoraphanin is the second major glucosinolate in black radish; its more important degradation product is sulforaphane, which has demonstrated to be an effective metabolite for diminishing hepatic cholesterol levels. One of the imbalances necessary for the formation of cholesterol gallstones is the biliary hypersecretion of this molecule towards the gallbladder (van Erpecum, 2011), a product of the high concentration of cholesterol in the hepatocytes; therefore, the inhibition of hepatic cholesterol is a key target for preventing biliary lithiasis.

A study evaluated glucoraphanin and sulforaphane in Syrian hamsters fed with a hypercholesterolemic diet for 7 weeks; these secondary metabolites diminished hepatic cholesterol levels, an effect that was associated with the inhibition of the genetic expression of Sterol Regulatory Element-binding Protein (SREBP-1 and SREBP-2) and Fatty Acid Synthase (Rodríguez-Cantú *et al.*, 2011), important molecules that regulate cholesterol and plant sterol homeostasis. The Syrian hamsters are important models for the study of cholesterol gallstones, because their bile has very similar components to that of human beings; consequently, the demonstrated effects indicate that under a lithogenic diet, both glucoraphanin and sulforaphane might prevent the formation of gallstones, since there is a decrease of hepatic cholesterol which could mean less secretion towards the bile; nevertheless, in the study, a hypercholesterolemic diet was administered that does not necessary cause the formation of gallstones. The possible action mechanism reported is critical, since in the study of gallstones the canalicular membrane proteins of the hepatocytes (ABCG5 and ABCG8) are the ones that have been more thoroughly analyzed; they are in charge of transporting the cholesterol from the liver towards the biliary canaliculus, but the cholesterol carriers and regulators inside the hepatocyte have been poorly investigated.

The SREBP genes are transcription factors that intervene in cholesterol homeostasis and biosynthesis inside the hepatocyte (Khesht and Hassanabadi, 2012). In C57L/J mice, susceptible to biliary lithiasis, the abnormal regulation of the SREBP-2 gene increases biliary cholesterol secretion; therefore, if we consider that glucosinolates inhibit this gene, we would expect a decrease in cholesterol biliary secretion. SREBP-2 is also an important modulator of the NPC1L1 protein, which is in charge of transporting biliary cholesterol and inhibiting its intestinal absorption (Pramfalk *et al.*, 2010). These precedents are an important factor, which must be investigated further in order to describe the action mechanism of glucosinolates and their degradation products with the same detail of other transport proteins and, at the same time, to promote the search of a preventive treatment that generates the least number of adverse effects.

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## CONCLUSION

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Black radish is an important cruciferous plant that has demonstrated hypocholesterolemic, antilithiasic and antioxidant properties in different biological models; these effects can prevent the formation of cholesterol gallstones, an increasingly prevalent gastrointestinal affection. The effects of black radish associated with the prevention of cholesterol gallstones may be due to two main glucosinolates, and to their degradation products: glucoraphasatin (raphasatin) and glucoraphanin (sulforaphane), but biological and phytochemical investigations must continue in order to complement the existent contributions.

## Conflict of Interest

The authors have declared that there is no conflicts of interest.

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