Research Article

Antilithiasic and Hypolipidaemic Effects of *Raphanus sativus* L. *var. niger* on Mice Fed with a Lithogenic Diet

Ibrahim Guillermo Castro-Torres,[1] Elia Brosla Naranjo-Rodríguez,[2] Miguel Ángel Domínguez-Ortíz,[3] Janeth Gallegos-Estudillo,[3] and Margarita Virginia Saavedra-Vélez[1]

[1] *Facultad de Química Farmacéutica Biológica, Universidad Veracruzana, Xalapa, Veracruz, Mexico*
[2] *Laboratorio de Neurofarmacología, Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, México, DF, Mexico*
[3] *Laboratorio de Productos Naturales, Instituto de Ciencias Básicas, Universidad Veracruzana, Xalapa, Veracruz, Mexico*

Correspondence should be addressed to Elia Brosla Naranjo-Rodríguez, eliab@unam.mx

In Mexico, *Raphanus sativus* L. *var. niger* (black radish) has uses for the treatment of gallstones and for decreasing lipids serum levels. We evaluate the effect of juice squeezed from black radish root in cholesterol gallstones and serum lipids of mice. The toxicity of juice was analyzed according to the OECD guidelines. We used female C57BL/6 mice fed with a lithogenic diet. We performed histopathological studies of gallbladder and liver, and measured concentrations of cholesterol, HDL cholesterol and triglycerides. The juice can be considered bioactive and non-toxic; the lithogenic diet significantly induced cholesterol gallstones; increased cholesterol and triglycerides levels, and decreased HDL levels; gallbladder wall thickness increased markedly, showing epithelial hyperplasia and increased liver weight. After treatment with juice for 6 days, cholesterol gallstones were eradicated significantly in the gallbladder of mice; cholesterol and triglycerides levels decreased too, and there was also an increase in levels of HDL ($P < 0.05$). Gallbladder tissue continued to show epithelial hyperplasia and granulocyte infiltration; liver tissue showed vacuolar degeneration. The juice of black radish root has properties for treatment of cholesterol gallstones and for decreasing serum lipids levels; therefore, we confirm in a preclinical study the utility that people give it in traditional medicine.

1. Introduction

Gallstones represent an important problem of public health; its prevalence is about 10% in middle-age persons and 20% in aged persons [1]. Cholesterol is the main component of the majority of gallstones [2]. This disease is strongly associated with atherosclerosis and metabolic syndrome [3], and its pharmacological treatment is limited, being cholecystectomy, an invasive surgical treatment, the only treatment for symptomatic gallstones [4]. Cholesterol gallstones formation is a complex process mediated by genetic and environmental factors [5, 6]. Many proteins (ATP-binding cassette (ABC)) are implicated in its formation, mainly the biliary lipids transporters ABCB4, ABCB11, ABCG5, ABCG8, ABCC7, and Niemann-Pick C1L1 protein, and these transporters are regulated in the liver by several transcription factors, including nuclear receptors farnesoid X receptor and liver X receptors [7–9]. Other important factors for gallstones formation are high levels of serum lipids (mainly cholesterol and triglycerides) [10]. New studies should focus on the more important proteins involved in biliary secretion and intestinal absorption of cholesterol, because those are key sites of cholesterol transport, on which new drugs might inhibit the formation of cholesterol gallstones; ezetimibe is a drug that showed potential benefit in cholesterol gallstone prevention, and ezetimibe inhibits the expression of Niemann-Pick protein in the small intestine, thereby decreasing the intestinal absorption of cholesterol [11].

There are pharmacological treatments, but there are also treatments based on traditional medicine; in Mexico, knowledge about the medicinal properties of plants is the basis for their use as home remedies. Black radish (*Raphanus sativus* L. *var niger*) is a plant belonging to the Brassicaceae family which contains a high concentration of glucosinolates [12]; experimental studies demonstrated that the aqueous extract and juice of this root possess pharmacological properties, mostly antioxidant, against urinary stones and for detoxifying enzyme activity [13–15]. In Mexican traditional medicine, black radish root has ethnopharmacological uses for the treatment of pigment and cholesterol gallstones and for decreasing serum lipids levels; juice squeezed from the root of black radish significantly decreases ($P < 0.05$) serum lipids levels in C57BL/6 mice fed with a lithogenic diet [16]; however, the active metabolites of black radish responsible for its therapeutic effects are unknown. The principal aim of this work was to evaluate the effects of the juice of black radish root in a biological model of cholesterol gallstones, in order to establish the scientific basis that explains its ethnopharmacological uses.

2. Material and Methods

2.1. Chemicals. Reagent grade cholesterol ($C_{27}H_{45}OH$) was purchased in Hycel of Mexico; reagent grade cholic acid ≥98% ($C_{24}H_{40}O_5$) purchased in Sigma-Aldrich; reagent grade β-cyclodextrin ($C_{42}H_{70}O_{35}$) H_2O 7.0 mol/mol purchased in Sigma Aldrich; ursodeoxycholic acid ($C_{24}H_{40}O_4$), Ursofalk 250 mg; sodium pentobarbital, Pfizer.

2.2. Plant Material. Fresh tubercles of black radish were collected in Veracruz State, Mexico, in 2011 and were used all experiments. The plant was classified taxonomically for the Instituto de Investigaciones Biológicas in Universidad Veracruzana. The voucher specimen of the plant (CIB 14655) is deposited at the herbarium for reference. The juice of black radish root was prepared in Laboratorio de Neurofarmacología in Facultad de Química. A fresh tubercle (557.7 g) was divided in six parts (60 g), and the juice was squeezed with extraction equipment Moulinex; from each part approximately 10 mL of juice were obtained.

2.3. HPLC Analysis. 500 mL of juice were lyophilized, and the dry material produced 4.5 g. The sample was treated using a previously described method [14]; however, we did not quantify glucosinolates of black radish root. Measurements were done on HPLC equipment (Agilent Technologies 1200 Series Binary SL), RP-C18 column (Zorbax Bonus 100 × 2.1 mm id, 3.5 μm). The mobile phase was 10 : 90 MeOH : H_2O with a flow rate of 2.0 mL/min.

2.4. Acute Toxicity Test. In the toxicity assay, we used a female mouse with the same characteristics of the mice of the main study (female C57BL/6 mice), according to the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals. We used a maximum of 7 animals with the following doses: control, vehicle (water), 175, 550, 1750, 2000, and 5000 mg/kg (0.1 mL/10 g body weight). Juice of black radish was lyophilized and was dissolved in purified water. The treatments were administered intragastrically. Neither food nor water was given up to 4 h after the treatment. Body weight, body weight change, signs of toxicity, behaviors, and mortality were observed during the first 4 hours. At the end of the experiment period, the sacrifice of female mice was performed after anesthesia (sodium pentobarbital) in order to avoid animal pain or stress.

2.5. Experimental Animals and Treatments. Thirty-six adult female C57BL/6 mice over 7–9 weeks of age and weighing 18–22 g were purchased in Harlan Laboratories of Mexico and were used in all experiments. Animal care and procedures were conducted according to the guidelines approved by Norma Oficial Mexicana (NOM-062-ZOO-1999) and were subjected to experimental protocols approved by the Programa Institucional para el Cuidado y Uso de Animales de Laboratorio, Facultad de Química, UNAM. All animals were housed in plastic cages in a temperature-controlled room with 12 : 12-h light-dark cycles and provided *ad libitum* access to food and water. Mice had free access to commercial rodent food (Purina Rat Chow). The animals were divided into 6 groups ($n = 6$), and treatments were administered intragastrically. One group was fed a normal diet (ND); five groups were also fed an experimental lithogenic diet containing 2% cholesterol and 0.5% cholic acid (10 g cholesterol/kg and 5 g cholic acid/kg) [17]. The vehicle group received purified water 0.1 mL/10 g (VEH). After feeding with lithogenic diet for 34 days, four groups were treated against cholesterol gallstones for 6 days: one group with ursodeoxycholic acid 0.5% in beta-cyclodextrin 2%, 0.1 mL/10 g (UDCA) and the other groups with juice of black radish root (JBR) diluted one hundredfold (1 : 100), tenfold (1 : 10) in purified water (1 : 100) and juice concentrate. All administrations were at doses of 0.1 mL/10 g of juice. During treatment against cholesterol gallstones (6 days), the VEH + LD group continued receiving lithogenic diet (40 days).

2.6. Gallbladder and Gallstones Analysis. Gallstones were usually visible through the gallbladder. To collect gallstones, the tip of the gallbladder was cut, the gallbladder was squeezed gently with forceps to remove stones, and the inside was washed with an isotonic saline solution to remove any gallstones adhering to the walls; the gallbladder mucosa was observed also. This process was carried out under a stereoscope and a dissecting microscope. The number of cholesterol gallstones in mice was counted.

2.7. Measurements of Total Cholesterol, HDL Cholesterol and Triglycerides. After being treated, all mice were weighed and anesthetized and had blood drawn from the retroorbital vein after an overnight fast. Sacrifice was performed after anesthesia (sodium pentobarbital) in order to avoid animal pain or stress. Serum was separated by centrifugation for further analysis. Determination of biochemical parameters was carried out using a photocolorimeter Dayton Randox.

2.8. Histological Evaluation of Gallbladder and Liver. Gallbladder and liver specimens were prepared and fixed in 4% neutral buffered formaldehyde embedded in paraffin, and 5 μm thin slices were cut and stained with haematoxylineosin. All histological sections were reviewed by a pathologist.

2.9. Statistical Analysis. Statistics were done using Sigma Stat Program. Statistical analyses were performed one-way ANOVA with Student-Newman-Keuls *post hoc*. Each point in the table and figure represents the mean ± standard deviation SD.

3. Results

3.1. HPLC Analysis. Our HPLC chromatogram did not prove the presence of glucosinolates; these results are probably due to how the sample was processed. The peaks on the HPLC chromatogram showed components with a retention time of less than 5, as can be seen in Figure 1. The results indicate that these compounds present in the juice of black radish root could be partly responsible for the therapeutic effects in mice.

3.2. Acute Toxicity Test. The results of toxicity test after testing the JBR are shown in Table 1. Single intragastric administration of JBR at all doses did not show visible signs of toxicity, abnormal behaviors, and mortality. Neither body weight was significantly changed relative to that of the control group during the first 4 hours. The juice can be considered bioactive and nontoxic ($LD_{50} = 0$; no toxicity).

3.3. Analysis of Gallstones. After 34 and 40 days, the lithogenic diet significantly induced the formation of gallstones in female mice and increased liver weight (Figure 2). In Figure 2(a) gallstones were usually visible and moved freely through the gallbladder. In Figure 2(b) an increase in liver weight in mice fed with a lithogenic diet was observed. Cholesterol gallstones were present in female mice as spherical aggregates (white/yellow) with diameters ranging between 0.4 and 0.8 mm; the gallbladders of the mice fed with a lithogenic diet were markedly expanded by the accumulation of bile fluid. The number of gallstones was compared between different experimental groups. In Figure 2(c) two groups of mice showed no cholesterol gallstones after treatment with the JBR concentrate and JBR 1/10. In gallbladders of the group treated with JBR 1/100 there were showen residues of cholesterol gallstones, while in the groups treated with UDCA 0.5% and LD, the cholesterol gallstones persisted within the gallbladder.

3.4. Cholesterol, HDL Cholesterol, and Triglycerides. The results from serum lipids analysis are shown in Table 2 and Figure 3. After 40 days, the mice of group LD + VEH had increased cholesterol and triglycerides levels; levels of HDL cholesterol decreased. The results from different experimental groups were compared with LD + VEH group. In Figure 3(a) after treatment with JBR, cholesterol levels
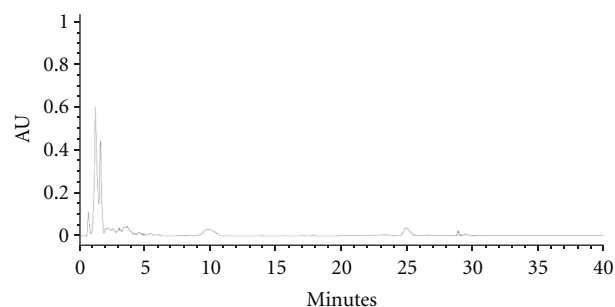

Figure 1: HPLC chromatogram of juice of black radish root.

Table 1: Toxicity test according to OECD guidelines.

Doses (mg/kg) of JBR	Mortality %	Signs of toxicity	Signs of toxicity %	Survival %
Female control	0	0/1	0	100
Vehicle (water)	0	0/1	0	100
175	0	0/1	0	100
550	0	0/1	0	100
1750	0	0/1	0	100
2000	0	0/1	0	100
5000	0	0/1	0	100

$LD_{50} = 0$; no toxicity.

decreased in mice, compared with mice fed with a lithogenic diet ($P < 0.05$). Although in two groups (UDCA 0.5% and JBR 1 : 10) gallstones did not remove the, those groups did show a decreased plasma cholesterol levels. In Figure 3(b) results of triglyceride levels are important, because concentration of JBR was crucial. Groups treated with JBR 1 : 10, and concentrated JBR had decreased levels of triglycerides to normal ranges; as the results presented in the graph of the group ND show, there was a significant difference. JBR 1 : 00 and UDCA 0.5% groups showed triglyceride levels above the normal range, significant difference in comparison with the LD + VEH group. In Figure 3(c) levels of HDL cholesterol decreased significantly in LD + VEH group, but in groups treated with BRJ and UDCA 0.5%, the levels of HDL cholesterol increased.

3.5. Microscopy Studies of Gallbladder Mucosa. All tissue of gallbladders was analyzed in optical microscope with 40x magnification. In Figure 4(a) there were no significant changes in the gallbladder mucosa in ND group; however, other experimental groups had significant changes when compared with the ND group. The groups with cholesterol gallstones (VEH and UDCA 0.5%) showed accumulation of biliary sludge, and its mucosas were the most damaged; these changes may be due to movement of gallstones and biliary sludge accumulation. In Figure 4(b) white arrows indicate cholesterol gallstones in VEH group, after fed for 40 days with a lithogenic diet. In Figure 4(c) Mucosa of group treated with UDCA 0.5%. In Figure 4(d) the groups treated with JBR showed less damage in mucosa of gallbladder.
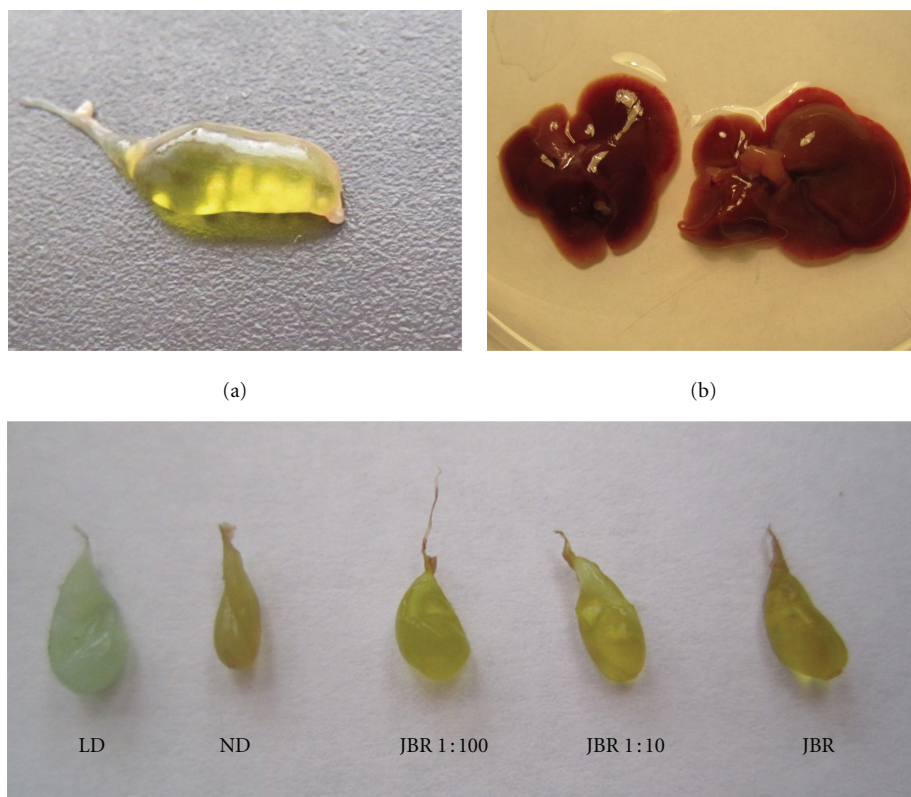
(a)

(b)

FIGURE 2: Cholesterol gallstones and liver in female mice.

TABLE 2: Black radish juice modifies some components in serum of mice.

Group	Biochemical parameter (mg/dL)		
	Cholesterol	HDL cholesterol	Triglycerides
ND	82 ± 2	67 ± 1.5	92.3 ± 0.8
LD + VEH	103.6 ± 3.5	51 ± 1.4	101.5 ± 1.2
LD + UDCA 0.5%	91 ± 2.4	60.5 ± 2	98.3 ± 1.3
LD + JBR 1 : 100	91.5 ± 2.2*	62.8 ± 0.7*	96.1 ± 0.1*
LD + JBR 1 : 10	80.6 ± 1.5*	62.8 ± 0.7*	93.8 ± 0.7*
LD + JBR	79.8 ± 1.8*	62.8 ± 1.1*	93 ± 0.6*

ND: normal diet; LD: lithogenic diet; VEH: vehicle; UDCA: ursodeoxycholic acid; JBR: juice of black radish root. Values are means ± SD using 6 mice in each experimental group. *Values indicate significant differences between groups treated with JBR ($P < 0.05$) versus LD + VEH group.

3.6. Histopathology Study. Histopathology results were compared between different experimental groups, and all tissue specimens were stained with hematoxilin eosin (Figure 5). There were no apparent histological changes in ND group. In Figure 5(a) Black arrows indicate the epithelial hyperplasia, and the white arrow indicates granulocyte infiltration in gallbladder of C57BL/6 mice after being fed for 40 days with a lithogenic diet (40x magnification). In Figure 5(b) the gallbladder of mice treated with JBR also presented inflammatory infiltration and epithelial hyperplasia, despite eradication of cholesterol gallstones (10x magnification). In Figure 5(c) all liver tissue specimens showed perivascular infiltration, vacuolar degeneration, and dilated central and portal veins (10x magnification).

4. Discussion

Our general aim was to evaluate the effectiveness of black radish juice against cholesterol gallstone disease and serum lipids (cholesterol, HDL cholesterol and triglycerides) in mice. The black radish contains high concentrations of a glucosinolates, but the exact mechanism of the biologically active compounds in black radish is not clear yet. Regarding possible active metabolites, our investigation proved that intact glucosinolates disappeared during processing and/or storage, for there were no peaks of glucosinolates on the HPLC chromatogram, as can be seen in Figure 1. There are two studies about HPLC analysis of black radish: in 1998 Lugasi and coworkers reported an HPLC chromatogram
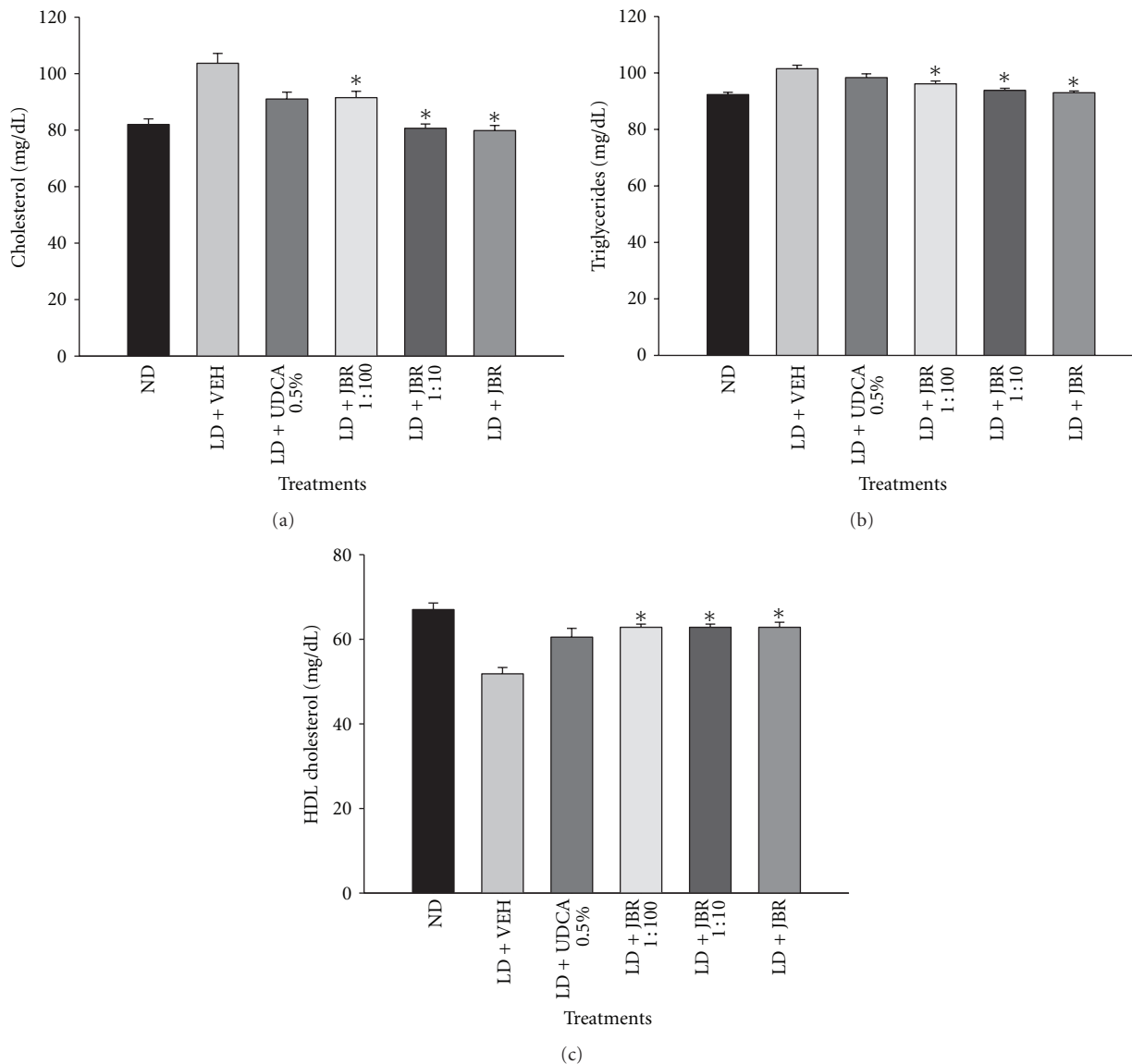
FIGURE 3: Effect of juice in serum lipids.

of juice squeezed of black radish, which did not detect presence of glucosinolates, only the peak corresponding to the internal standard (benzyl glucosinolates) [18]. Hanlon and coworkers (2007) reported an HPLC chromatogram of desulfoglucosinolates in an aqueous extract of black radish [14]. Both authors performed a quantification of secondary metabolites in black radish by HPLC. In our analysis we did not quantify secondary metabolites. In the HPLC chromatogram of Hanlon and coworkers, degradation products of glucoraphasatin were the most apparent; also observed were degradation products of glucoraphanin; these results are very important, because glucoraphanin and its degradation product, sulforaphane, have been shown to have a potential hypocholesterolemic effect in animal models [19]. It is likely that compounds related to glucoraphanin are responsible for lowering cholesterol and triglycerides in the serum of mice in our study although we must emphasize

that the hypocholesterolaemic effects do not necessarily cause the dissolution of cholesterol gallstones in mice. A hypolipidaemic effect is directly related to the prevention of gallstones, rather than to treatment, but it is important to know that the reduction of plasma cholesterol is a direct consequence of the decrease in intestinal absorption although we did not evaluate this. When absorption is decreased, biliary cholesterol secretion can be decreased, thereby reducing the saturation of this lipid in the vesicle, which favors the activity of bile salts on cholesterol gallstones, since they are the major solutes of bile. An important aspect was that we did not evaluate the elimination of cholesterol in feces, as neutral sterol, because, by demonstrating the reduction of plasma cholesterol, there must have been be a route of elimination, or accumulation. The toxicity test was undertaken prior to the therapeutic evaluation of the juice of black radish root; there is currently a tendency to
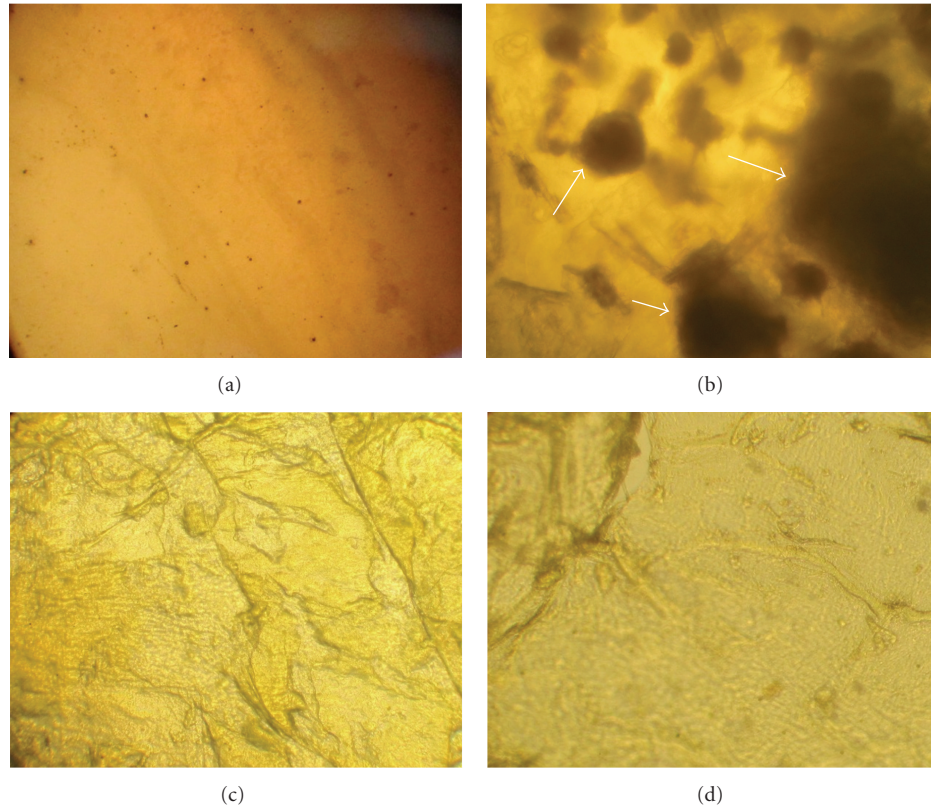
(a)

(b)

(c)

(d)

FIGURE 4: Microscopic analysis in mucosa of gallbladder.
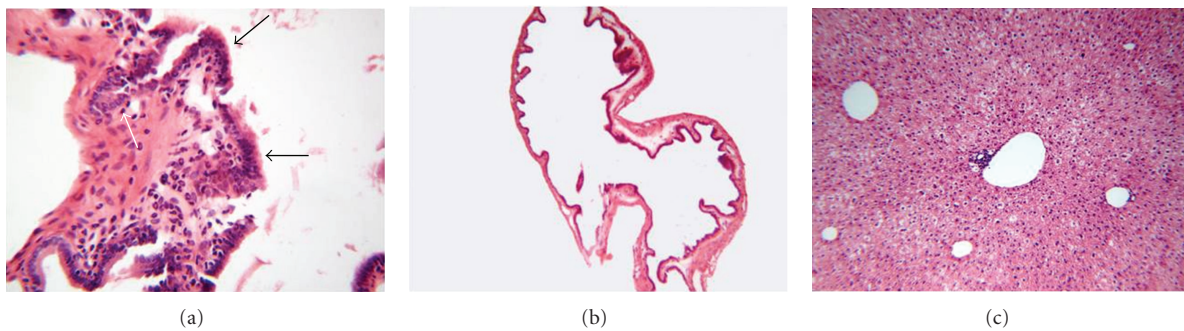

(a)

(b)

(c)

FIGURE 5: Histopathological analysis of gallbladder and liver.

call not using laboratory animals in toxicological tests, due to the high cost and the suffering imposed on the animals. We used the toxicity test according to OECD guidelines for minimizing the number of animals required to estimate the acute oral toxicity of juice black radish. In addition to the estimation of LD_{50} and confidence intervals, the test allows the observation of signs of toxicity. According to OECD guideline and Lipnick et al. [20], substances that explain the median lethal dose (LD_{50}) higher than 5 g/kg body weight by oral route can be considered practically nontoxic. Thus, it can be concluded that juice black radish is absent of the acute oral toxicity. Although the exact mechanism of the biologically active compounds in black radish is not clear yet, a beneficial effect of the drug was evident against cholesterol

gallstones and both cholesterol and triglyceride levels. Our study is new and represents part of the verification of the uses that are given to the plant in Mexican traditional medicine. Our mice developed cholesterol gallstones after 34 days of being fed with a lithogenic diet; Ebihara and Kiriyama (1985) reported similar observations in male ICR mice also using a high cholesterol and cholic acid diet [21]. We did not determine the concentrations of cholesterol, bile salts, and phospholipids in the hepatic bile, as is usually done in studies to inhibit the formation of cholesterol gallstones, because we did not do a preventative treatment; on the contrary, we administered a treatment to destroy gallstones when they were already formed. The juice of black radish root diluted tenfold and concentrated juice decreased cholesterol and

triglycerides levels and also removed cholesterol gallstones in mice. The juice diluted one hundredfold did not remove all gallstones in mice; the effect of concentration was determinant. In another study related to this work, the juice of black radish root exhibited significant antioxidant properties in rats fed with a diet rich in lipids (20% sunflower oil, 2% cholesterol, 0.5% cholic acid in normal chow); the juice was diluted tenfold with water and the animals drank it instead of water for 9 days [11]. These results are related to cholesterol gallstones formation; the rats were fed a diet rich in lipids, but rats have no gallbladder in which gallstones can be formed or accumulated; therefore, this diet is a lithogenic diet for mice, because formation of gallstones was only produced by simultaneous feeding of cholesterol, cholic acid, and fats. This might lead us to think that it also produced antioxidant effects in our mice. The experimental group treated with ursodeoxycholic acid had decreased levels of cholesterol and triglycerides; however, in this group gallstones was not removed. This is logical because treatment with ursodeoxycholic acid requires a long time to dissolve cholesterol gallstones, and we only administered ursodeoxycholic acid for 6 days. Ursodeoxycholic acid has been recommended as the first-line pharmacological therapy in a subgroup of symptomatic patients with small, radiolucent cholesterol gallstones and its longterm administration has been shown to promote the dissolution of cholesterol gallstones [22]. The effect to decreasing lipid levels could also be enhanced by the administration of beta-cyclodextrin in ursodeoxycholic acid, because it is a cyclic oligosaccharide that binds cholesterol and bile acids *in vitro*, and it has been previously shown to be an effective plasma cholesterol-lowering agent in hamsters and domestic pigs and also produces antilithiasic effects in hamsters [23]. The lithogenic diet produced granulocyte infiltration in mice; according to data reported in the literature, the presence of gallstones in gallbladder produces neutrophilic infiltration and reactive epithelial changes (increased nucleus-to-cytoplasm ratio, mitoses, and prominent nucleoli) that can easily be confused with neoplasia [24]. After the administration of treatments against the effects of the lithogenic diet, the gallbladders of mice continued to show granulocyte infiltration and hyperplasia in the epithelium of gallbladder. Although changes in the gallbladder mucosa were different between treatment groups and the vehicle group, the histopathological changes were similar.

We consider this paper as an important contribution because we showed antilithiasic effect in mice, which is different from demonstrating an antilithogenic effect that prevents the formation of gallstones. There are currently few studies about molecular mechanisms that destroy gallstones; therefore, it is important to start studying which processes could lead to create antilithiasic effects. Our work did not determine the molecular mechanism that destroys gallstones, but it is a background for continuing the experimental studies about the action mechanism of phytochemical components in black radish. It is understandable that medical science is more interested in the prevention of cholesterol gallstones disease, because as there are many risk factors for developing it; but there are many people who suffer from gallstones now, and they require a treatment. Drug therapy for cholesterol gallstone disease plays a limited role, but novel interesting information about the molecular mechanisms responsible for the formation of gallstones is now available [25]. Unfortunately, for symptomatic gallstones, the ideal therapy is cholecystectomy, which is an invasive method [26]. Thanks to the knowledge of Mexican traditional medicine, we demonstrated an antilithiasic effect of black radish juice in mice; in addition we demonstrated decreasing serum cholesterol and triglycerides, two important lipids involved in cholesterol gallstones formation.

5. Conclusion

Juice squeezed from black radish root has properties against cholesterol gallstones and for decreasing serum lipids levels. Considering that it is a non-toxic natural product, more studies should be made to find the action mechanism of secondary metabolites.

Conflict of Interests

The authors declare that they have no conflicts of interests.

Acknowledgments

References

[1] P. Portincasa, A. Moschetta, and G. Palasciano, "Cholesterol gallstone disease," *Lancet*, vol. 368, no. 9531, pp. 230–239, 2006.

[2] C. E. Ruhl and J. E. Everhart, "Gallstone disease is associated with increased mortality in the United States," *Gastroenterology*, vol. 140, no. 2, pp. 508–516, 2011.

[3] N. Méndez-Sánchez, J. Bahena-Aponte, N. C. Chávez-Tapia et al., "Strong association between gallstones and cardiovascular disease," *American Journal of Gastroenterology*, vol. 100, no. 4, pp. 827–830, 2005.

[4] A. Di Ciaula, D. Q. H. Wang, H. H. Wang, L. Bonfrate, and P. Portincasa, "Targets for current pharmacologic therapy in cholesterol gallstone disease," *Gastroenterology Clinics of North America*, vol. 39, no. 2, pp. 245–264, 2010.

[5] K. J. Maurer, M. C. Carey, and J. G. Fox, "Roles of infection, inflammation, and the immune system in cholesterol gallstone formation," *Gastroenterology*, vol. 136, no. 2, pp. 425–440, 2009.

[6] C. S. Stokes, M. Krawczyk, and F. Lammert, "Gallstones: environment, lifestyle and genes," *Digestive Diseases*, vol. 29, no. 2, pp. 191–201, 2011.

[7] K. J. Van Erpecum, "Pathogenesis of cholesterol and pigment gallstones: an update," *Clinics and Research in Hepatology and Gastroenterology*, vol. 35, no. 4, pp. 281–287, 2011.

[8] M. C. Vázquez, A. Rigotti, and S. Zanlungo, "Molecular mechanisms underlying the link between nuclear receptor function and cholesterol gallstone formation," *Journal of Lipids*, vol. 2012, Article ID 547643, 7 pages, 2012.

[9] I. G. Castro-Torres, "Cholesterol gallstones formation: new scientific advances," *Revista GEN (Gastroenterología Nacional)*, vol. 66, no. 1, pp. 57–62, 2012.

[10] A. H. M. Smelt, "Triglycerides and gallstone formation," *Clinica Chimica Acta*, vol. 411, no. 21-22, pp. 1625–1631, 2010.

[11] O. de Bari, B.A. Neuschwander-Tetri, M. Liu M et al., "Ezetimibe: its novel effects on the prevention and the treatment of cholesterol gallstones and nonalcoholic fatty liver disease," *Journal of Lipids*, vol. 2012, Article ID 302847, 16 pages, 2012.

[12] A. Lugasi, A. Blázovics, K. Hagymási, I. Kocsis, and Á. Kéry, "Antioxidant effect of squeezed juice from black radish (*Raphanus sativus* L. *var niger*) in alimentary hyperlipidaemia in rats," *Phytotherapy Research*, vol. 19, no. 7, pp. 587–591, 2005.

[13] R. Vargas, R. M. Perez, S. Perez, M. A. Zavala, and C. Perez, "Antiurolithiatic activity of *Raphanus sativus* aqueous extract on rats," *Journal of Ethnopharmacology*, vol. 68, no. 1–3, pp. 335–338, 1999.

[14] P. R. Hanlon, M. G. Robbins, L. D. Hammon, and D. M. Barnes, "Aqueous extract from the vegetative portion of Spanish black radish (*Raphanus sativus* L. *var. niger*) induces detoxification enzyme expression in HepG2 cells," *Journal of Functional Foods*, vol. 1, no. 4, pp. 356–365, 2009.

[15] P. R. Hanlon, D. M. Webber, and D. M. Barnes, "Aqueous extract from Spanish black radish (*Raphanus sativus* L. *var. niger*) induces detoxification enzymes in the HepG2 human hepatoma cell line," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 16, pp. 6439–6446, 2007.

[16] I. G. Castro-Torres and E. B. Naranjo-Rodríguez, "Treatment with black radish (*Raphanus Sativus* L. *var niger*) reduces serum lipids levels in mice fed with a lithogenic diet," *Revista Latinoamericana de Quimica*, vol. 39, no. 3, p. 273, 2012.

[17] E. Reihnér and D. Ståhlberg, "Lithogenic diet and gallstone formation in mice: integrated response of activities of regulatory enzymes in hepatic cholesterol metabolism," *British Journal of Nutrition*, vol. 76, no. 5, pp. 765–772, 1996.

[18] A. Lugasi, A. Blázovics, K. Hagymási et al., "Antioxidant and free radical scavenging properties of squeezed juice from black radish (*Raphanus sativus* L. *var niger*) root," *Phytotherapy Research*, vol. 12, pp. 502–506, 1998.

[19] L. N. Rodríguez-Cantú, J. A. Gutiérrez-Uribe, J. Arriola-Vucovich, R. I. Díaz-De La Garza, J. W. Fahey, and S. O. Serna-Saldivar, "Broccoli (*brassica oleracea* var. *italica*) sprouts and extracts rich in glucosinolates and isothiocyanates affect cholesterol metabolism and genes involved in lipid homeostasis in hamsters," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 4, pp. 1095–1103, 2011.

[20] R. L. Lipnick, J. A. Cotruvo, R. N. Hill et al., "Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures," *Food and Chemical Toxicology*, vol. 33, no. 3, pp. 223–231, 1995.

[21] K. Ebihara and S. Kiriyama, "Prevention of cholesterol gallstones by a water-soluble dietary fiber, konjac mannan, in mice," *Nutrition Reports International*, vol. 32, no. 1, pp. 223–229, 1985.

[22] P. Portincasa, A. Di Ciaula, H. H. Wang, A. Moschetta, and D. Q. H. Wang, "Medicinal treatments of cholesterol gallstones: old, current and new perspectives," *Current Medicinal Chemistry*, vol. 16, no. 12, pp. 1531–1542, 2009.

[23] N. Boehler, M. Riottot, J. Férézou et al., "Antilithiasic effect of *β*-cyclodextrin in LPN hamster: comparison with cholestyramine," *Journal of Lipid Research*, vol. 40, no. 4, pp. 726–734, 1999.

[24] D. E. Hansel, A. Maitra, and P. Argani, "Pathology of the gallbladder: a concise review," *Current Diagnostic Pathology*, vol. 10, no. 4, pp. 304–317, 2004.

[25] D. Q. Wang, D. E. Cohen, and M. C. Carey, "Biliary lipids and cholesterol gallstone disease," *Journal of Lipid Research*, vol. 50, supplement, pp. S406–S411, 2009.

[26] V. I. Reshetnyak, "Concept of the pathogenesis and treatment of cholelithiasis," *World Journal of Hepatology*, vol. 4, no. 2, pp. 18–34, 2012.