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Effect of Tannins from *Quercus suber* and *Quercus coccifera* Leaves on Ethanol-Induced Gastric Lesions in Mice

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The gastroprotective effects of 70% acetone extracts of *Quercus suber* and *Quercus coccifera* leaves and of tannins (pedunculagin, castalagin, phillyraeoidin A, and acutissimin B) purified from these extracts were examined in the mouse using the ethanol-induced gastric ulcer model. Both extracts (25, 50, and 100 mg/kg), given orally, prevented the formation of ethanol-induced lesions in the stomach. The percent protection varied between 68 and 91%. Purified tannins (50 mg/kg) were also effective in protecting the stomach against ethanol, and the percent protection varied from 66 to 83%. Castalagin was the most potent. Both extracts and all of the tannins tested (10, 25, and 50 μg/mL) strongly inhibited (55−65%) the lipid peroxidation of rabbit brain homogenate. These results suggest that the gastroprotective effects of extracts of *Q. suber* and *Q. coccifera* leaves and the purified tannins in this experimental model are related to their anti-lipoperoxidant properties.

KEYWORDS: Experimental gastric ulcer; lipid peroxidation; medicinal plants; *Quercus* sp.; tannins; castalagin

INTRODUCTION

Various polyphenolic compounds, including tannins (oligomeric hydrolyzable tannins and complex tannins) and other metabolites, have been isolated from medicinal plants, and their biological and pharmacological activities were reviewed by Okuda et al. (*1*). Their biological activities include antisecretory and antiulcerogenic effects (*1*−*3*). Crude extracts of some medicinal plants that are rich in tannins are traditionally used worldwide to treat gastric ulcers. *Quercus* sp. are some of the most important tannin-containing plants (*4*). *Q. ilex* (leaves, roots, and bark) is used in Algerian folk medicine to treat gastritis and gastric ulcers, and the tannins in these plants are thought to be responsible for the medicinal effects. Tannic acid and phenolic acids in plant extracts are reported to protect the stomach against various necrotizing agents (*3*, *5*−*8*). It was shown that an aqueous extract of *Q. ilex* root bark is gastroprotective (50.8%) when given orally to rats at a dose of 18 mg of total polyphenols/kg (*7*). An acetone extract of *Q. ilex* is also protective against absolute ethanol-induced gastric lesions when given at 50 mg of total polyphenols/kg; a 10-fold lower dose was inefficient (*8*). This cytoprotective effect is thought to be due to the antisecretory effect of phenolic acids, which directly inhibit the proton pump (*5*, *6*). Moreover, pentagalloylglucose isolated from *Paeoniae radix* also inhibits H^+,K^+-ATPase and might be responsible for the inhibition of acid secretion that this plant causes (*9*). Besides their beneficial effects, tannins are inhibitors of enzyme such as pepsin in the stomach (*10*) by a precipitating effect (*11*). Another effect that may impair gastric defense is the interaction of tannins with mucin (*12*).

The mechanism of oxidant injury involves the generation of membrane lipid peroxides (*13*). Many authors have reported a close relationship between lipid peroxidation and gastric mucosal lesions (*14*−*18*). Tannins and other polyphenols were reported to be very potent antioxidants and to inhibit lipid peroxidation in different assay systems (*1*, *19*, *20*).

Quercus species contain large amounts of tannins; for example, *Q. robur* contains ∼10% (w/w) hexahydroxydiphenoyl esters (ellagitannins), the main compounds in this class being castalagin and vescalagin (*4*). A number of polyphenolic compounds were recently isolated and identified from the leaves of Algerian *Q. suber* and *Q. coccifera*, including pedunculagin, casuarictin, tellimagrandin I, tellimagrandin II, castalagin, vescalagin, phillyraeoidin E, acutissimin B, mongolicain A, (+)-catechin, quercetin, quercitrin, and kaempferol 3-*O*-(6″-*O*-galloyl)-β-D-glucopyranoside (*21*).

The aim of this study was to examine the gastroprotective effect of tannins recently isolated from *Q. suber* and *Q. coccifera*, which have not been tested for their antiulcer effect

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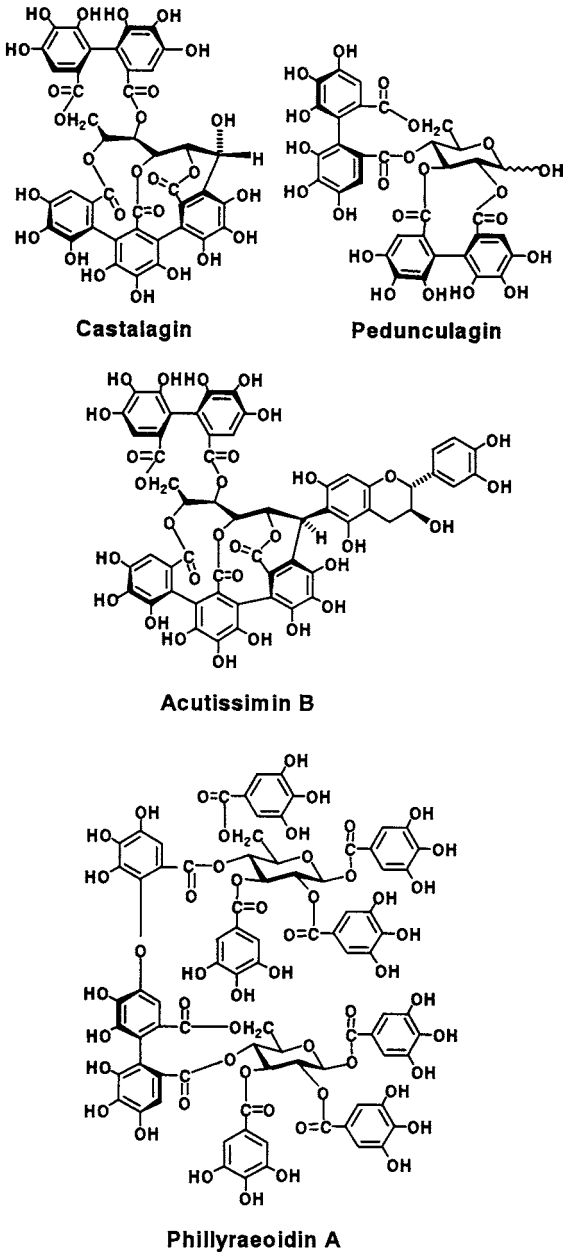

Figure 1. Structures of tannins purified from *Q. suber* and *Q. coccifera*.

in mice. Because ulcer development involves in great part free radicals (*16*), the ability of these tannins to inhibit lipid peroxidation was tested using rabbit brain homogenate. Crude acetone extracts of both *Quercus* species were included for comparison.

MATERIALS AND METHODS

Plant Materials. *Q. suber* and *Q. coccifera* leaves were collected from EL-Kala National Park (Algeria). Plant species were identified at the Laboratory of Phytosociology (University Ferhat Abbas, Setif, Algeria), and voucher specimens were deposited at the laboratory. The leaves were air-dried at room temperature and then finely powdered (100 mesh). The powder was extracted with 70% aqueous acetone overnight and filtered. The filtrate was evaporated under reduced pressure at <40 °C. The aqueous residue was lyophilized and stored in a desiccator at −20 °C.

Chemicals. Acutissimin B, castalagin, pedunculagin, and phillyraeoidin A (**Figure 1**) were purified from the *Q. suber* and *Q. coccifera* leaves as previously reported (*21*). The purity of each tannin used in the present study was confirmed by NMR and HPLC (reversed and normal phases) analyses.

Animals. Animals (mice and rabbits) were fed standard diets. Male mice weighing 20−30 g (Institut Pasteur, Algiers, Algeria) were used in the gastric ulceration experiments. The mice were fasted for 20 h but allowed free access to water until 1 h before the experiment. During the fasting period, the animals were placed three per cage with wide-mesh wire bottoms to prevent coprophagy. Rabbits (local strain) weighing 1.5−2.0 kg were used for lipid peroxidation experiments after a 1 week period of stabilization at the animal house.

Ethanol-Induced Gastric Ulcer. The aqueous acetone extracts of *Q. suber* and *Q. coccifera* leaves and purified tannins were suspended in 5% carboxymethyl cellulose (CMC) at appropriate concentrations. Plant extracts, tannins, and ethanol were administered to mice by the intragastric route (0.1 mL/20 g of body weight). In the first experiment, the animals received *Quercus* acetone extracts (25, 50, and 100 mg/kg) 1 h before 40% ethanol was administered. In the second experiment, the animals received 50 mg/kg of one of the four purified tannins in the same manner; *Q. coccifera* and *Q. suber* extracts were included for comparison. The control groups received the same volume of CMC instead of the test solution.

The animals were sacrificed by cervical dislocation 15 min after the ethanol gavage. The stomachs were removed and filled with 1 mL of 5% formalin for 10 min after ligation of both the esophageal and pyloric ends. The stomachs were opened along the greater curvature, pinned flat on a corkboard, and washed gently with saline. Using a color CCD camera (DIC-D, World Precision Instruments, Sarasota, FL), stomach images were coded and stored in a PC for subsequent analysis of the lesions, which was performed by a person blind to the treatments using the UTHSCA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, TX, and available from the Internet by anonymous FTP from maxrad6.uthsca.edu). Lesions were delimited manually, and the resulting pixel area was converted to square millimeters by ImageTool using an internal conversion factor. The number and total area of the lesions were determined for each stomach.

Lipid Peroxidation Assay. Levels of the thiobarbituric acid-reactive substances (TBARS) in rabbit brain homogenate were measured according to the method of Ohkawa et al. (*22*). Rabbits were anesthetized with urethane (1.2 g/kg ip), and blood was withdrawn from the animal by perfusing a sufficient volume of cold saline via the jugular vein. The brain was immediately removed and homogenized in ice-cold 1.15% KCl (7 g of tissue/100 mL). To induce lipid autoxidation, 5 mL of the brain homogenate was incubated for 1 h at 37 °C in a shaking water bath. The test substances were suspended in 5% CMC and added (125 μL) to the homogenate at final concentrations of 10, 25, and 50 μg/mL. The same volume of CMC was added to the control tubes (100% lipid peroxidation rate). The incubated solutions were then centrifuged (4000 rpm, 10 min). The supernatant (2 mL) was added to 0.5 mL of 8.1% SDS and 4 mL of 0.8% TBA dissolved in 20% acetic acid. The mixture was heated in a water bath at 100 °C for 1 h. After cooling with tap water, the tubes were centrifuged for 10 min at 4000 rpm and the absorbance of the supernatant was read at 532 nm against a blank solution prepared without TBA. The relative peroxidation rate was calculated by comparing the absorbance of the test supernatant with that of the control.

Statistical Analysis. The results are given as means ± SEM. Statistical significance was determined using a one-way analysis of variance followed by Dunnett's test for multiple comparisons with the control. The level of significance was fixed at 5%.

RESULTS

Effect of *Q. suber* and *Q. coccifera* Leaf Extracts on Ethanol-Induced Gastric Lesions. Treating mice with 40% ethanol for 15 min caused severe gastric mucosal hemorrhagic lesions. The average number of lesions was 45.0 ± 13.9 covering 12.1 ± 3.0 mm^2 of the mucosa. Pretreatment with both *Quercus* extracts reduced the number of lesions (pooled mean ± SEM = 11.7 ± 3.6). The resulting area of lesions was significantly reduced with all three doses tested (**Figure 2**). The percent protection of the stomach ranged from 68 to 91%, and

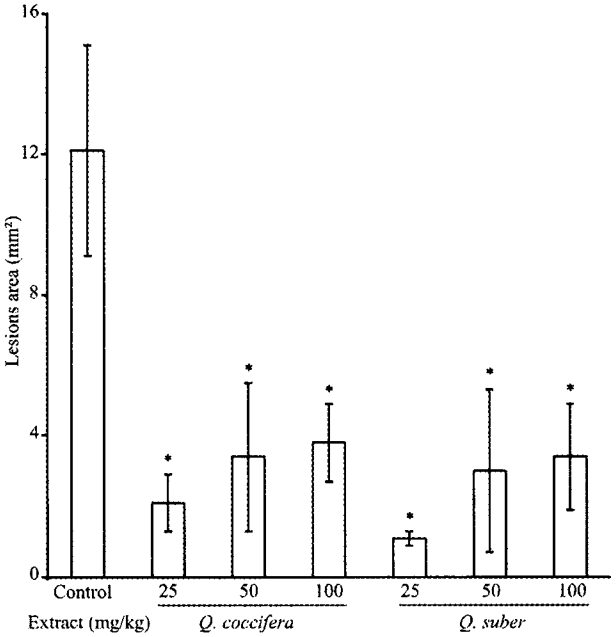

Figure 2. Effects of aqueous acetone extracts of *Q. suber* and *Q. coccifera* leaves on ethanol-induced gastric lesions in the mouse. Test solutions were given by orogastric instillation 1 h before 40% ethanol was administered. Results are expressed as the mean ± SEM (n = 8–10). * $P < 0.05$ versus the control.

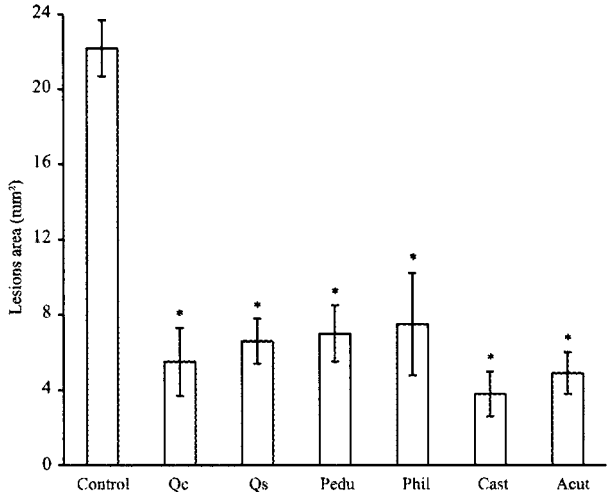

Figure 3. Effect of extracts of *Q. suber* (Qs) and *Q. coccifera* (Qc) and of pedunculagin (Pedu), phillyraeoidin A (Phil), castalagin (Cas), and acutissimin B (Acu) on ethanol-induced gastric lesions in the mouse. Test solutions (50 mg/kg) were given by orogastric instillation 1 h before 40% ethanol was administered. Results are expressed as the mean ± SEM (n = 8–10). * $P < 0.05$ versus the control.

the level of protection was independent of the origin of the extract ($P > 0.05$). Neither acetone extract produced dose-dependent protection ($P > 0.05$); the pooled mean areas of the lesions were 3.1 ± 1.5 mm² for the *Q. coccifera* extract and 2.5 ± 1.6 mm² for the *Q. suber* extract.

Effect of Purified Tannins on Ethanol-Induced Gastric Lesions. The effects of both *Quercus* extracts and of tannins isolated and purified from these plants (50 mg/kg) against gastric lesions induced by 40% ethanol were tested. Pretreatment of mice with these extracts or tannins (pedunculagin, phillyraeoidin A, castalagin, and acutissimin B) significantly decreased the total surface lesions ($P < 0.05$; **Figure 3**). There were no differences between the molecules tested ($P > 0.05$). The extracts of *Q. coccifera* and *Q. suber* were as potent as the pure molecules; the calculated percent protection varied between 66 and 83%.

Table 1. Effects of Extracts of *Q. suber* and *Q. coccifera* Leaves and of Pedunculagin, Castalagin, Acutissimin B, and Phillyraeoidin A on Lipid Peroxidation of Rabbit Brain Homogenate[a]

compound	concn (μg/mL)	relative peroxidation (%)	% inhibition
Q. suber extract	10	41.8 ± 4.9*	58.2
	25	40.5 ± 5.5*	59.5
	50	41.2 ± 4.7*	58.8
Q. coccifera extract	10	36.4 ± 7.0*	63.6
	25	35.9 ± 7.6*	64.1
	50	35.2 ± 7.2*	64.8
acutissimin B	10	35.4 ± 4.2*	64.6
	25	34.5 ± 4.4*	65.5
	50	36.0 ± 4.3*	64.0
castalagin	10	45.1 ± 4.2*	54.9
	25	46.1 ± 5.5*	53.9
	50	47.1 ± 5.6*	52.9
pedunculagin	10	38.3 ± 6.9*	61.7
	25	36.9 ± 7.1*	63.1
	50	36.2 ± 6.4*	63.8
phillyraeoidin A	10	38.9 ± 3.9*	61.1
	25	37.6 ± 4.1*	62.4
	50	36.1 ± 4.2*	63.9

[a] Results are expressed as the mean ± SEM (n = 5). *$P \leq 0.5$ versus the control (100% peroxidation).

Effects of Purified Tannins on Lipid Peroxidation. The aqueous acetone extracts of *Quercus* leaves strongly inhibited the lipid peroxidation caused by rabbit brain homogenate, as did the purified tannins. The observed inhibition was not significantly related to the concentration ($P > 0.05$). As shown in **Table 1**, the average inhibition induced by *Q. coccifera* extract (64.2%) was slightly greater than that induced by *Q. suber* extract (58.8%; $P > 0.05$). Of the four tannins tested, castalagin had the lowest rate of inhibition (53.9 vs 63.4%). Comparisons made between *Quercus* extracts and the purified molecules did not show any significant differences.

DISCUSSION

The oral administration of ethanol to mice caused severe gastric lesions after 15 min. Pretreating the animals with 25, 50, or 100 mg/kg of lyophilized 70% acetone extract of *Q. suber* and *Q. coccifera* leaves 1 h before the ethanol treatment reduced the occurrence of mucosal lesions. Because these extracts contain appreciable amounts of polyphenols, as measured by the Prussian blue assay (*Q. suber*, 318 mg/g; *Q. coccifera*, 342 mg/g), these polyphenols may be responsible for this gastroprotective activity. Neither *Quercus* species extract showed any dose−response effect; however, the mean lesions area tended to increase with increasing administered dose. This observation suggests that the lowest used dose (i.e., 25 mg/kg) gave a maximal response, and no further significant effect could be observed. The absence of a dose−effect response may be also due to the complex composition of the extracts. It is possible that some compound(s) present in the extract may antagonize the effect of some other compounds (e.g., tannins) when their dose is increased above a threshold. An extract of *Q. ilex* leaves is reported to be effective in protecting the rat stomach against 50% ethanol (*7*). Moreover, a *Q. coccifera* extract reduced the severity of ethanol-induced gastric damage in rats with a high curative ratio reaching 99% (*23*). Tannins from medicinal plants have been reported to possess gastroprotective properties (*2*, *3*, *8*). Therefore, among tannins purified from both *Q. suber* and

Q. coccifera, representative tannins of different types, ellagitannin monomer (pedunculagin), dimer (phillyraeoidin A), *C*-glucosidic tannin (castalagin) and complex tannin (acutissimin B), were examined in this study for their gastroprotective activity in mouse. It appears that all types of tannins (50 mg/kg) tested can prevent the formation of ethanol-induced gastric lesions when administered 1 h before the necrotizing agent. The percent protection afforded by these substances was very high; it reached 82.8% with castalagin. Tannic acid, a standard tannin, was shown to protect the rat stomach against the noxious effect of ethanol (*7*). Other tannins and related polyphenols show gastroprotective activity in various gastric ulcer models (*2*−*5*).

It has long been recognized that gastric damage induced by a gastric barrier disrupting agent (e.g., ethanol) is followed by the disappearance of acid from the gastric lumen (*24*). Acid disappearance has been attributed to back-diffusion of the acid through the disrupted gastric barrier (*25*). Acid in the gastric lumen is one of the main factors leading to cell disruption (*26*). Phenolic acids, such as tannic acid and ellagic acid, reduce gastric acid secretion (*2*) by inhibiting the proton pump (*6*). Pentagalloylglucose isolated from *P. radix* also inhibits membrane H^+,K^+-ATPase and might be responsible for the inhibition of acid secretion caused by this plant (*9*). The gastroprotective effect of the tannins tested might be due to the inhibition of acid secretion before the administration of ethanol to mice.

The antioxidant properties of tannins are also proposed as a mechanism for their gastroprotective activity (*17*). In rats subjected to water immersion restraint stress, gastric mucosal lesions developed in a time-dependent manner with increased lipid peroxide levels (*16*). A strong relationship between lipid peroxidation and ulcer formation in the stomach has been observed elsewhere (*14*, *17*, *18*, *27*). Hong et al. (*28*) tested the effect of 25 tannins and related compounds and found catechin to be one of the most potent polyphenols at inhibiting lipid peroxidation. In this study, the effect of *Quercus* extracts and purified tannins on lipid autoperoxidation was examined using rabbit brain homogenate. *Q. suber* and *Q. coccifera* extracts inhibited lipid peroxidation by >58%. A relatively small concentration (10 μg/mL) was as effective as 50 μg/mL. This effect is probably due to the presence of polyphenolic compounds and tannins in these extracts. Gallic acid, tannic acid, quercetin, and catechin (10 μg/mL) caused 49−65% inhibition of brain homogenate lipid peroxidation (data not shown). Tannins purified from *Quercus* were also effective in inhibiting lipid peroxidation; 10 μg/mL inhibited peroxidation by >54%. A large molecular size having many phenolic hydroxyl groups appears to be important for exhibiting the inhibitory effect as shown by acutimissin B and phillyraeoidin A. In all of the tested conditions, there was no significant correlation between the concentration of the compounds and the inhibition of lipid peroxidation. Similarly, Grinberg et al. (*29*) reported the potent anti-lipid peroxidation of purified tea polyphenols in a red blood cells system. As in the present results, this effect was maximal with a concentration as low as 10 μg/mL. Ellagitannins, active constituents of medicinal plants, inhibit lipid peroxidation (*19*). The inhibition caused by ellagitannins is stronger than that of other tannins with similar structures (*30*). The tannin fraction and procyanidins lower plasma lipid peroxides and conjugated dienes in rats and mice (*31*, *32*).

In conclusion, the gastroprotective properties of *Quercus* extracts and isolated tannins (pedunculagin, castalagin, phillyraeoidin A, and acutissimin B) might be partly related to their strong antiperoxidant activity. Further investigations of these pure molecules are needed to examine their possible involvement in reinforcing the gastric barrier (mucus and acid secretion, gastric blood flow, and epithelium repair).

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