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Effect of Some Phenolic Compounds and *Quercus* Tannins on Lipid Peroxidation

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Abstract: In the present study, the anti-lipoperoxidant activity of some phenolic acids, flavonoids and purified tannins from Quercus sp. was determined. Phenolic acids (10, 25 and 50 µg/ ml) inhibited lipid peroxidation by 42 – 51%. Caffeic acid seems to be the most potent inhibitor among phenolic acids. Tannic acid, a commercially available tannin, inhibited lipid peroxidation in the same manner as phenolic acids. The inhibition did not exceed 43%.Catechin and quercetin significantly inhibited lipid peroxidation at all tested concentrations and the percentages of inhibition varied between 38 and 60%. However, flavonoid glycosides (rutin and diosmin) did not behave in the same way. *Quercus* purified tannins showed high inhibition of lipid peroxidation. The observed inhibition was not significantly related to the concentration. Among the four tested tannins, castalagin gave the smallest rate of inhibition (53.9%) versus acutissimin B (65.5%).

Key words: Lipid peroxidation · Polyphenols · Antioxidants · *Quercus* tannins

INTRODUCTION

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals" [1]. Free radicals have been implicated in cell damage and the pathogenesis of at least 50 diseases [2].

Free radicals are produced in the body as part of normal metabolism; for example, superoxide and nitric oxide have important physiological functions. In general, free radicals are highly reactive and can attack membrane lipids, generating a carbon radical, which in turn reacts with oxygen to produce a peroxyl radical which may attack adjunct fatty acids to generate new carbon radicals. This process leads to a chain reaction forming lipid peroxidation products [2].

The reactive oxygen species generated by the metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells contribute to gastric mucosal damage [3]. The process of lipid peroxidation is mediated by the interaction of hydroxyl radicals with the cell membrane producing lipid-derived free radicals like conjugated dienes and lipid hydroperoxides which are highly reactive products that cause oxidative damage [3].

Lipid peroxidation occurs in he body by the oxidation of unsaturated fatty acids. This process could be enzymatic or non-enzymatic event. It occurs in three steps initiation, propagation and termination stage [4]. During the initiation step, one atom of hydrogen is extracted from the unsaturated fatty acid (LH) by hydroxyl radical ($^{*}OH$) resulting in the formation of lipid radical (L^{*}) leading to the formation of conjugated diene. The propagation step is characterized by the reaction of conjugated diene with oxygen to form peroxyl radical (LOO.) which in turn attack another unsaturated fatty acid to form unstable hydroperoxide (LOOH) and a new radical. In the termination step, a reaction between two of the formed radicals occurs to form non-radical products [4].

In the body, endogenous antioxidant mechanisms exist to limit the formation and to scavenge free radicals. These include antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase and catalase. SOD prevents the accumulations of O_2 radical by converting it to H_2O_2, which has lower toxicity (2). H_2O_2 is metabolised to H_2O by either glutathione peroxidase or catalase [5]. In addition to antioxidant enzymes, some other molecules in the body act as antioxidants like glutathione. Other exogenous products act as antioxidants and free radical scavenging molecules like, vitamin E and C and plant derived natural antioxidants like carotenoids and polyphenols [6].

An increase of lipid peroxidation was observed in patients with peptic ulcer disease[7]. Lipid peroxidation in the gastric mucosa was reported to be associated with ulcer genesis in different experimental gastric ulcer models. Lipid peroxidation (as thiobarbituric acid reactive

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substances) in the rat gastric mucosa was increased by 50% ethanol [8, 9]. Moreover, the increase in lipid peroxidation in the gastric mucosa is involved in the pathogenesis of gastric injury induced by water-immersion restraint stress [10]. Free radicals were suggested to be involved in the pathogenesis of pylor legation induced ulcers in rats, an increase in the level of lipid peroxidation in the stomach after pyloric legation was observed [11]. This level of peroxidation was also increased in the rat stomach by water-immersion and this increase was inhibited by the administration of antioxidants [12]. In addition, lipid peroxidation mediated by oxygen radicals plays an important role in the mechanism of ulcer aggravation induced by indomethacin [13].

Quercus species (Fagaceae) are widely distributed in Algeria. *Q. ilex* roots bark is used in the Algerian folk medicine to treat gastropathies. Other *Quercus* species are also used in traditional medicine [14]. Polyphenols and tannins present in these plants could be responsible for activity probably by acting as antioxidants and inhibitors of lipid peroxidation. In this study, antilipoperoxidant activity of some phenolic acids, flavonoids and purified tannins was determined.

MATERIAL AND METHODS

Plant Material: Plant samples used in these studies were collected from different places in the East of Algeria. *Quercus ilex* (leaves and root bark) was collected from the National Park of Babor. *Quercus suber, Quercus faginea* and *Quercus coccifera* leaves were collected from El Kala National Park. Each plant species was identified at the laboratory of Phytosociology, Department of Biology University Ferhat Abbass, Sétif Algeria. Voucher specimens were deposited at the laboratory.

The plant samples were air dried in shadow and finely powdered in a rotating knife grinder. The powder was sieved through a 1 mm mesh to remove large fragments. Each plant powder was then used for the extraction procedure.

Animals: Rabbits (local strain) were used as a source of lipids for lipid peroxidation experiments after one week-period of adaptation at the animal house.

Polyphenols Extraction Procedures: Polyphenols from different *Quercus* species were extracted in different ways according to the study performed. Polyphenols were extracted according to their solubility in organic solvents. Acetone (70%) was used since it gives the higher yields compared to ethanol. Tannins (Fig. 4) were purified from *Q. suber* and *Q. coccifera* leaves using 70% acetone as described in Ito *et al*. [15].

Determination of Total Polyphenols in Plant Extracts: Total polyphenols were determined in plant extracts at different occasions using two methods. The Prussian bleu method using tannic acid or gallic acid as standards[16] [17].

Determination of Lipid Peroxidation Rate: Lipid peroxidation was induced in the rabbit brain homogenate incubated at 37°C. The measurement of TBARS in the homogenate was carried out in the presence of test solutions at different concentrations and compared to the values of TBARS measured in the absence of test solutions (100% peroxidation).

The effect of phenolic compounds and purified tannins on lipid peroxidation was tested. The level of thiobarbuteric acid reactive substances (TBARS) in rabbit brain homogenate was measured according to the method of Ohkawa *et al*. [18]. Animals were anaesthetized with 25 % urethane (1.2 g/kg i.p.) and the blood was withdrawn from the animal by perfusing 30 ml of cold saline via the jugular vein. The brain was immediately removed and homogenized in ice cold 1.15% KCl. To induce autoxidation, a 7% brain homogenate was incubated for 1 h at 37°C in a shaking water bath. The test solutions (10, 25 and 50 µg/ml) were incubated with the homogenate. The control tubes contained 5% CMC instead of test solutions. The incubated solutions were then centrifuged for 10 min at 4000rpm and an aliquot of 2 ml was added to 0.5 ml of 8.1% SDS and 4 ml 0.8% thiobarbuteric acid (TBA) dissolved in 20% acetic acid. The tubes were then shaked and 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. The absorbance of the supernatant was measured at 532 nm against a blank solution incubated without TBA. The absorbance of the control tubes was used as maximum peroxidation rate (100%). Results were expressed as percentage of relative peroxidation in relation to maximum rates.

RESULTS AND DISCUSSION

Phenolic acids (10, 25 and 50 µg/ ml) inhibited lipid peroxidation in the rabbit brain homogenate by 42 – 51%. Caffeic acid seems to be the most potent inhibitor of lipid peroxidation among phenolic acids (Fig.1). No differences were noticed between concentrations of each tested compound. Tannic acid, a commercially available tannin,

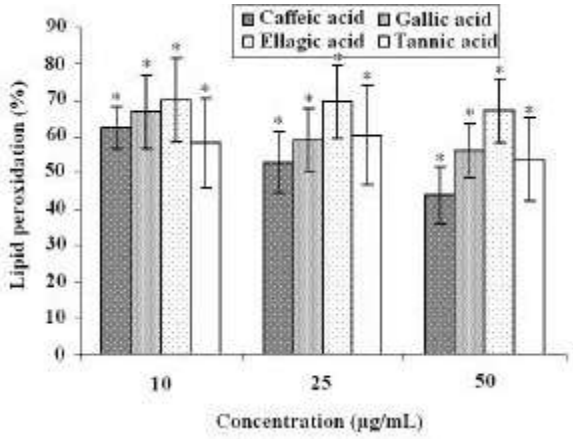

Fig. 1: Effects of phenolic acids on lipid peroxidation in the rabbit brain homogenate. Results are expressed as mean±SEM (n = 5). *P= 0.5 with respect to control (100 % peroxidation).

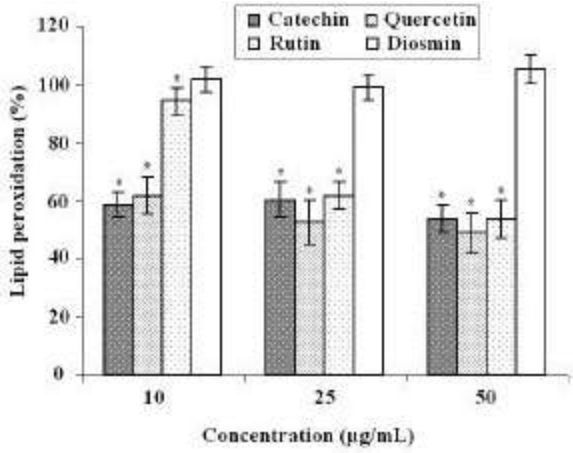

Fig. 2: Effects of flavonoids on lipid peroxidation in the rabbit brain homogenate . Results are expressed as mean± SEM (n = 5). *P= 0.5 with respect to control (100 % peroxidation)

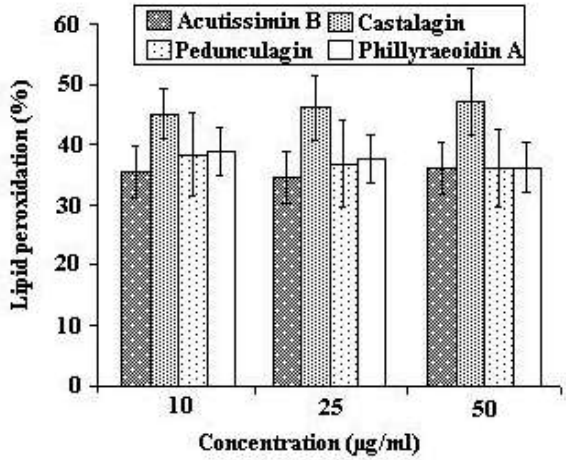

Fig. 3: Effects of isolated tannins: pedunculagin, castalagin, acutissimin B and phillyraeoidin A on lipid peroxidation of the rabbit brain homogenate. Results are expressed as mean±SEM (n = 5). *P= 0.5 with respect to control (100% peroxidation).

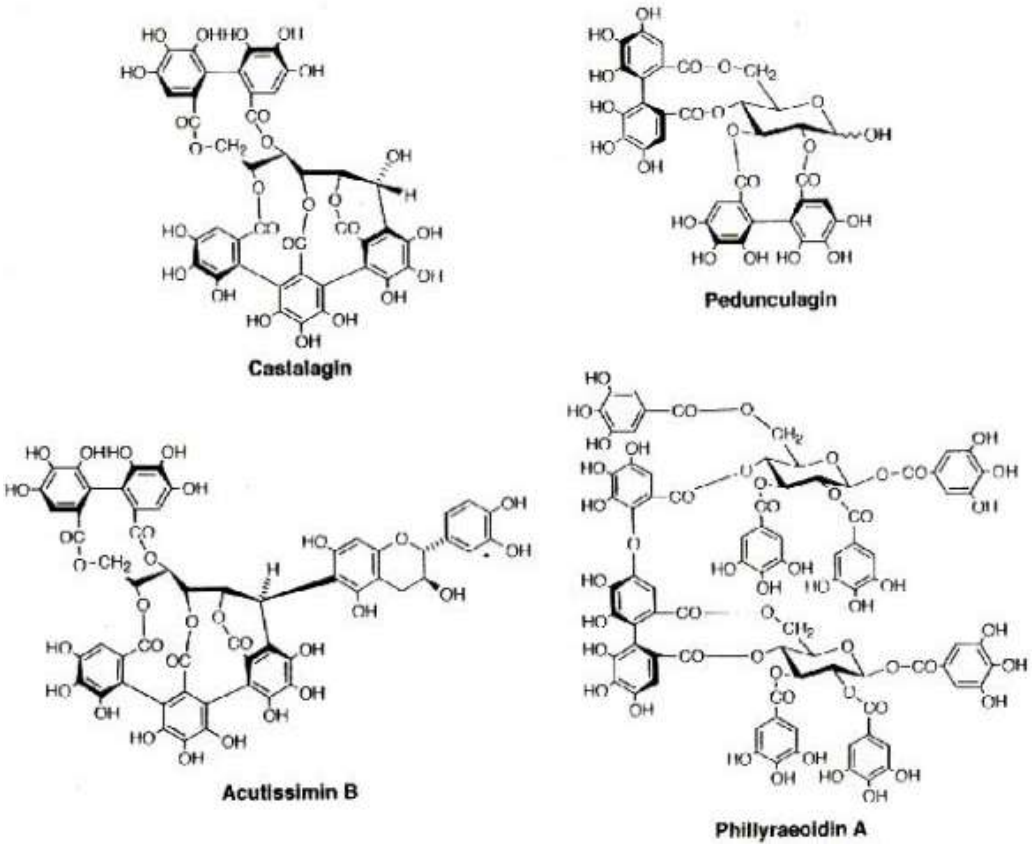

Fig. 4: Structures of tannins: 1- castalagin, 2- pedunculagin, 3- acutissimin B and 4- phillyraeoidin A

inhibited lipid peroxidation in the same manner as phenolic acids. The inhibition did not exceed 43% (Fig.1). Catechin and quercetin significantly inhibited lipid peroxidation at all tested concentrations and the percentages of inhibition varied between 38 and 60%(Fig. 2). However, flavonoid glycosides (rutin and diosmin) did not behave in the same way. Rutin 25 and 50 µg/ml inhibited lipid peroxidation by approximately 40%. Paradoxically, rutin (10 µg/ml) did not show inhibitory effect on lipid peroxidation. Diosmin did not affect this proccess at all concentrations (Fig. 2).

Oxygen-derived free radicals including superoxide and hydroxyl radicals are involved in the pathogenesis of tissue injury initiating and promoting lipid peroxidation. Polyunsaturated fatty acids of the cellular membranes are degraded by lipid peroxidation with subsequent disruption of membrane integrity, suggesting that lipid peroxidation mediated by oxygen radicals is an important cause of the damage and destruction of cell membranes [19, 20]. When the generation of oxygen radicals exceeds the ability of tissue antioxidants to detoxify them, the resultant oxidative stress can cause damage. Because ROS (particularly the hydroxyl radical) are unstable, for their short half-lives in biological systems they tend to react with nearly macromolecules such as lipids and proteins. These reactions damage and denature the macromolecules via peroxidation processes [21].

A strong relation between lipid peroxidation and ulcer formation in the stomach has been observed [22]. The tissue damage in the stomach induced by ethanol is associated with the generation of reactive oxygen species. Ethanol induces oxidative stress, increased xanthine oxidase activity and lipid peroxidation in the gastric mucosa [23]. Xanthine oxidase is a source of oxygen free radicals. This enzyme reacts with molecular oxygen, thereby releasing superoxide free radicals. Flavonoids inhibit xanthine oxidase activity, thereby resulting in decreased oxidative injury [24]. Free radical scavenging activity and the antilipoperoxidant activity could be proposed mechanisms of the gastroprotective action of these compounds. flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical. Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive. Tannins and flavonoids isolated from

medicinal herbs were reported to exhibit free radical scavenging activities toward DPPH. Increase of galloyl groups, molecular weight and ortho-hydroxyl structure enhanced the activity of tannins; whereas, the number and position of hydroxyl groups were important features for the scavenging of free radicals by flavonoids[26,27].

Flavonoids and other phenolics act as antioxidants and inhibit lipid peroxidation [28] and therefore protect the stomach from oxidative damage induced by ethanol [29]. Furthermore, phenolics have been reported to directly inhibit lipid peroxidation [30]. The determination of the antilipoperoxidant activity of pure phenolic molecules may be one way to explain the gastroprotective properties of these extracts and compounds reported previously[14]. The effect of purified tannins and pure phenolics on lipid autoperoxidation was examined.

Pure molecules, like gallic acid, tannic acid, quercetin and catechin (10 µg/ml) cause 49-65% inhibition of brain homogenate lipid peroxidation. Tannins purified from *Quercus* species were also effective in inhibiting lipid peroxidation, 10 µg/ml inhibited the peroxidation by more than 54%. There was no significant correlation between the concentration of the compounds and the inhibition of lipid peroxidation and 50% inhibition seem to be a maximum response. This could be attributed to the method of determination itself or to maximum inhibition by afforded by the lowest concentration of polyphenolic compound. Similarly, Grinberg *et al*. [31] 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 micrograms/ml. Hong *et al*. [32] tested the effect of 25 tannins and related compounds and found that catechin is one of the most potent polyphenol in inhibiting lipid peroxidation. Ellagitannins as active constituents of medicinal plants exert inhibitory activity on lipid peroxidation[33]. This inhibition is exhibited more strongly by ellagitannins than by the other types of tannins of similar structure[34]. Tannin fraction and procyanidins were shown to lower plasma lipid peroxides and conjugated dienes in rats and mice[35] and to have a strong DPPH free radical scavenging activities [26]. It is possible therefore that phenolic acids, flavonoids and purified tannins examined in this study exhibit gastroprotective properties by acting as inhibitors of lipid peroxidation process. phenolic acids, flavonoids and purified tannins showed antilipoperoxidant activity except for diosmin. This antioxidant property is believed to be responsible (at least in part) for the gastroprotective effects of *Quercus* tannins and phenolic compounds.

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