Antioxidant Effects in the Quinone Fraction from *Auxemma oncocalyx* TAUB.

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In previous studies in vitro we showed that the quinone fraction (QF) from the heartwood of *Auxemma oncocalyx* TAUB. presented antiplatelet and antioxidant activities. In the present work, the QF antioxidant property was evaluated in models of CCl₄-induced hepatotoxicity in rats, and prolongation of pentobarbital-induced sleeping time in mice. Our results showed that levels of plasma glutamate-pyruvate-transaminase (GPT), as well as glutamate-oxalate-transaminase (GOT), were increased by the administration of CCl₄. On the other hand, only GPT levels were reduced by the QF treatment. Pentobarbital sleeping time was prolonged by the administration of CCl₄ and reduced by the QF treatment. Moreover, QF did not alter the pentobarbital-induced sleeping time. In conclusion, we showed that QF, represented mainly by oncocalyxone A, has hepatoprotective activity, and this effect is at least in part due to the antioxidant activity of this quinone.

**Key words** *Auxemma oncocalyx*; antioxidant property; hepatoprotective effect

*Auxemma oncocalyx* TAUB. belongs to the Boraginaceae family. It is popularly known as “pau branco” and occurs frequently in the state of Ceará, Northeast Brazil. The stem bark of the tree is an astringent and is used in the treatment of wounds. β-Sitosterol, its glycoside (3β-D-glucopyranosylsitosterol) and allantoin, probably responsible for the related popular use, were isolated from the plant. Besides these compounds, at least seven quinones were also isolated from the heartwood. Recently, three anthraene derivatives, auxenone, oncocalyxonol and auxemim, were also isolated. Quinones are organic compounds that are considered to be products from phenol oxidation. Similarly, quinone reduction can originate the corresponding phenols. It’s lactic activity is responsible for the therapeutic utilization of plants containing quinones. Several naphthoquinones present antileishmanial activity, and others are responsible for bactericide, fungicide and antitumoral activities present in extracts from *Kigelia pinnata* DC. Benzquinones and naphthoquinones showed activity against trypanosomatidies, while other studies showed their in vitro activity against trypanomatidies from *Trypanosoma cruzi*, and also in vitro antitumoral, antileishmanial and antimalarial activities. Other pharmacological studies revealed that the stem alcoholic extract from *A. oncocalyx* presents analgesic and anti-edematogenic, antitumoral, antiprotein and antiplatelet activities. Recently, the in vitro antiplatelet activity of the quinone fraction from the heartwood of *A. oncocalyx* and the cytotoxicity against human cells in vitro of oncocalyxones A and C isolated from that part of the plant, were demonstrated. Several plant extracts as well as their isolated chemical constituents are known to present antioxidant activities. Besides, hepatoprotective effects have been reported in plant flavonoids, such as silymarin extracted from the milk thistle, *Silybum marianus* L. G.AERT. This property has been related to the capacity of this flavonoid to prevent reduced glutathione depletion, induced by several hepatotoxic substances. Moreover, flavonoids have been reported to act as stabilizing biological membranes, and it is possible that the silymarin hepatoprotective effect is, at least in part, due to an action at the membrane level, although the effect of silymarin on plasma membrane could be explained by its free radical scavenging action. In previous studies in vitro, we showed that the quinone fraction from *A. oncocalyx* presented a potent platelet antiaggregant activity, and it was also capable of inhibiting lipoperoxidation in rat brain homogenates, as determined by the decrease of thiobarbituric acid reactive substances (TBARS) production and chemiluminescence emission. The objectives of the present work were to investigate possible hepatoprotective and antioxidant activities of the quinone fraction from *A. oncocalyx* in two different experimental models in rodents.

**MATERIALS AND METHODS**

**Plant Material and Fractionation** The plant was collected at the city of Pentecoste, state of Ceará, in the northeastern region of Brazil, and identified by Prof. A. G. Fernandes from the Biology Department of Federal University of Ceará. The plant voucher (no. 18459) is deposited at the Prisco Bezerra Herbarium of the Federal University of Ceará. The quinone fraction (QF) was prepared from a ground heartwood methanolic extract, through exhaustive aqueous extraction followed by lyophilization. The hydrosoluble components contained approximately 80% of oncocalyxone A, and this compound was isolated and its chemical structure determined according to the method previously described.

**Test Animals** Male Wistar rats (180—230 g) and male Swiss mice (25—30 g) were obtained from the Animal House of the Federal University of Ceará. Animals were maintained in an air-conditioned room at 23—25°C, 12 h light—12 h dark, and fed a standard laboratory diet and tap water ad libitum. Experiments were performed according to

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the guide for the care and use of laboratory animals from the Department of Health and Human Services of the United States of America.

**Drugs** Carbon tetrachloride (CCl₄) was purchased from Merck, Germany. Pentobarbital sodium was from Sigma, U.S.A., and kits for measurement of the activities of the enzymes glutamate-pyruvate-transaminase (GPT) and glutamate-oxalate-transaminase (GOT) were from Labtest, Brazil. All other drugs were of analytical grade.

**Hepatoprotective Study** Hepatic injury in rats was induced by the subcutaneous administration of CCl₄/olive oil (1:1 v/v, 3 ml/kg) as described by Lin et al. Rats were divided into four groups of eight animals each. Group 1 was the control group, which received the vehicle used for QF dissolution (saline, 10 ml/kg, i.p.). Quinone fraction 10 or 30 mg/kg, i.p., was administered to groups 3 and 4, 30 min before and 24 or 48 h after CCl₄ administration. The remaining group received CCl₄ only. All animals were anesthetized with ether, then blood was collected from the orbital plexus in tubes containing heparin, 72 h after CCl₄ administration. Blood samples were centrifuged at 3000 rpm at room temperature for 5 min for plasma separation. The activities of plasma GPT and GOT were measured according to the method described by Reitman and Frankel.

**Modification of Pentobarbital-Induced Sleeping Time** The effects of quinone fraction on pentobarbital-induced sleeping time, and of this fraction on CCl₄-induced prolongation of pentobarbital sleeping time, were studied in mice, as described by Gilani et al. Mice were separated into groups of ten animals each (treated with QF or controls). To test the direct effect of QF, doses of 10 and 30 mg/kg, i.p., and doses of 10, 30, 100 and 200 mg/kg, p.o., were given as single doses. Pentobarbital (40 mg/kg, i.p.) was administered 30 or 60 min after intraperitoneal and oral treatments respectively. Control animals of the groups treated intraperitoneally received saline (10 ml/kg). At QF doses higher than 30 mg/kg orally administered, the vehicle was 2% DMSO in saline, and this vehicle was used for the control groups.

To assess the effect of QF on CCl₄-induced prolongation of pentobarbital sleeping time, doses of 1, 10 and 30 mg/kg, i.p., and doses of 10, 30, 100 and 200 mg/kg, p.o., were given as single doses. CCl₄ 20% in olive oil (3 ml/kg, s.c.) was administered 30 or 60 min after intraperitoneal and oral treatment, respectively. Pentobarbital (40 mg/kg, i.p.) was given 24 h after CCl₄ administration. Control animals of the groups treated intraperitoneally received saline (10 ml/kg) instead of QF. In the case of oral administration, the vehicle was 2% DMSO in saline (used for controls). One more group was added to the study, and received saline (10 ml/kg, i.p.) or 2% DMSO in saline (10 ml/kg, p.o.), respectively, followed by pentobarbital.

**Statistical Analysis** Data were analyzed by one-way analysis of variance (ANOVA), followed by the Tukey–Kramer test for multiple comparisons.

**RESULTS**

**Effect of QF on CCl₄-Induced Hepatotoxicity** The results of QF on the liver injury model in rats are summarized in Table 1. They indicated that 72 h after CCl₄ administration there were significant increases of plasma GTP and GOT levels, as compared to the control group (p<0.001). However, GTP and GOT levels in the groups receiving QF 10 and 30 mg/kg, i.p., were lower compared with the CCl₄-treated control, but this inhibition was significant for GPT levels only. The values of GPT level inhibition for QF doses of 10 and 30 mg/kg were very similar (27, 29% respectively), indicating that the dose of 10 mg/kg probably produced the maximum effect.

**Effect of QF on Pentobarbital-Induced Sleeping Time** The results of QF on pentobarbital-induced sleeping time in mice are shown in Tables 2 and 3. In both cases, after intraperitoneal and oral administration, QF at the doses tested did not produce significant alterations of pentobarbital-induced sleeping time compared to the control group (p<0.005).

**Effect of QF on CCl₄-Induced Prolongation of Pentobarbital Sleeping Time** The results of QF on CCl₄-induced prolongation of pentobarbital sleeping time in mice are presented in Tables 4 and 5. They indicate that CCl₄ injection produced significant increases in pentobarbital-induced sleeping time compared to the controls of groups receiving QF intraperitoneally or orally (p<0.001). However, the intraperitoneal treatment with QF doses resulted in decreases in pentobarbital-induced sleeping time compared to the group that received CCl₄ only. The inhibition was signifi-
Table 3. Effect of the Orally Administered Quinone Fraction (QF) from *Auxemma oncocalyx* TAU on Pentobarbital-Induced Sleeping Time in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Sleeping time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% DMSO in saline 10 ml/kg, p.o. + pentobarbital 40 mg/kg, i.p.)</td>
<td>29.13 ± 2.23 (18)</td>
</tr>
<tr>
<td>QF 10 mg/kg, p.o. + pentobarbital</td>
<td>27.97 ± 2.42 ns (10)</td>
</tr>
<tr>
<td>QF 30 mg/kg, p.o. + pentobarbital</td>
<td>27.13 ± 2.40 ns (10)</td>
</tr>
<tr>
<td>QF 100 mg/kg, p.o. + pentobarbital</td>
<td>26.31 ± 1.77 ns (9)</td>
</tr>
<tr>
<td>QF 200 mg/kg, p.o. + pentobarbital</td>
<td>29.28 ± 1.85 ns (9)</td>
</tr>
</tbody>
</table>

Male Swiss mice (25—30 g) were used. Animals were treated with saline (control) or QF once, 60 min before pentobarbital injection. After pentobarbital administration, the sleeping time (in minutes) was registered. Values are expressed as means ± S.E.M. of the number of animals in parenthesis. ns = not significant (One-way ANOVA and Tukey–Kramer as post hoc test).

Table 4. Effect of the Intraperitoneally Administered Quinone Fraction (QF) of *Auxemma oncocalyx* TAU on Carbon Tetrachloride (CCl4)-Induced Prolongation of Pentobarbital Sleeping Time in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Sleeping time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline 10 ml/kg, i.p. + pentobarbital)</td>
<td>29.54 ± 2.03 (10)</td>
</tr>
<tr>
<td>Saline, i.p. + CCl4 20% in olive oil, 3 ml/kg, s.c. + pentobarbital 40 mg/kg, i.p.</td>
<td>132.57 ± 11.16* (9)</td>
</tr>
<tr>
<td>QF 1 mg/kg, i.p. + CCl4 + pentobarbital</td>
<td>109.52 ± 10.30 (9)</td>
</tr>
<tr>
<td>QF 10 mg/kg, i.p. + CCl4 + pentobarbital</td>
<td>73.06 ± 7.98** (10)</td>
</tr>
<tr>
<td>QF 30 mg/kg, i.p. + CCl4 + pentobarbital</td>
<td>74.22 ± 6.41** (9)</td>
</tr>
</tbody>
</table>

Male Swiss mice (25—30 g) were used. Control animals received saline and pentobarbital. Treated groups received saline or QF once, 30 min before CCl4 injection. Pentobarbital was administered 24 h after the treatment with CCl4, and then the sleeping time (in minutes) was registered. Values are expressed as means ± S.E.M. of the number of animals in parenthesis. *p < 0.001 vs. control and **p < 0.05 vs. the group treated with 2% DMSO in saline + CCl4 + pentobarbital (One-way ANOVA and Tukey–Kramer as post hoc test).

Discussion

According to the literature, quinones are among those plant constituents that present antioxidant activity. The oil as well as a quinone derivative isolated from *Nigella sativa* seeds, a species popularly used for the treatment of rheumatism and other inflammatory diseases, were tested as possible inhibitors of eicosanoid synthesis and membrane lipid peroxidation. Both compounds inhibited the cyclooxygenase and lipoxygenase pathways of the arachidonic acid metabolism in rat leukocytes, and also inhibited the non-enzymatic peroxidation in rat brain phospholipid liposomes.29)

According to Tripathi et al.,40) antaarquione and naphthoquinone isolated from the root ethanolic extract of *Rubia cordifolia* LINN (Rubiaceae) were capable of inhibiting the lipid peroxidation induced by cumene hydroperoxide in rat liver homogenate. Recently, Bezabih et al.41) showed that other quinones, such as isofuranonaphthoquinones isolated from the roots of *Bulbine capitata*, present antioxidant activity. Also, an anthocyanin derivative from soybean cotyledons showed scavenger activity against ascorbyl and lipid radicals in vitro.42)

In the present work, we studied the antioxidant activity of the quinone fraction isolated from the heartwood of *Auxemma oncocalyx* in the rat model of CCl4-induced hepatotoxicity, in which both free radicals and liperoxidation are involved.43,44) It is assumed that CCl4 is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical. This free radical may react again with oxygen to form a trichloromethylperoxyl radical which is even more reactive than the trichloromethyl radical, and elicits lipid peroxidation, causing the disruption of Ca2⁺ homeostasis and finally cell death.45,46) Moreover, lipid peroxidation is one of the primary events of CCl4-induced liver damage.47) Therefore, leakage of large quantities of enzymes into the blood stream are often associated with massive necrosis of the liver.48)

The experimental intoxication induced by carbon tetrachloride is widely used for modeling liver injury in rats. Hepatotoxicity is connected with a severe impairment of cell protection mechanisms. The localization of the injury is defined mainly by the biotransformation of CCl4, which is cytochrome P-450 dependent. Free radicals initiate the process of lipid peroxidation, which is generally the cause of inhibition of enzyme activity.49,50) In the assessment of liver dam-
age by CCl₄ hepatotoxicity, the determination of enzyme levels such as GOT and GPT is largely used. GOT is present in the liver, heart, muscle, kidney and brain, and it catalyzes the conversion of aspartate to oxaloacetate and glutamate. Necrosis or membrane damage releases the enzyme into circulation; therefore, it can be measured in serum. High levels of GOT indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. GPT catalyzes the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, GPT is more specific to the liver, and is thus a better parameter for detecting liver injury. It has been shown that anthocyanins derived from Bulgarian red wine, administered for a 5-day period, protect the liver from the toxic injury caused by CCl₄, as measured by the increase in serum transaminase activities. Acute parenchymal injury leads to a marked activation of GOT and GPT.

Our results using the model of CCl₄-induced hepatotoxicity in rats demonstrated that QF caused significant inhibition only of GPT levels. The doses tested produced similar effects, and maximum inhibition was 29% observed with the dose of 30 mg/kg, relative to the CCl₄ treated group. It has been shown that rat pretreatment with aqueous extracts of four traditional Chinese medicines used for hepatic disturbances presented hepatoprotective effects as demonstrated by the decrease in serum GOT and GPT levels after CCl₄-induced hepatotoxicity, relative to the group treated with CCl₄ only.

In addition, treatment with QF significantly reduced the CCl₄-induced prolongation of pentobarbital sleeping time in mice, confirming its hepatoprotective effect. The doses of 10 and 30 mg/kg, i.p., produced similar effects, and maximum inhibition of 45% was observed with the dose of 10 mg/kg relative to the group treated only with CCl₄. Thus, QF was more potent after intraperitoneal administration. Species differences probably explain the more intense hepatoprotective effect of QF observed in mice. It is known that enzymatic and hepatocyte injuries induced by CCl₄ impair the process of biotransformation of drugs by the liver. The principle behind the barbiturate-induced prolongation of sleeping time is that if liver microsomal enzymes have been destroyed by disease or a hepatotoxin, the metabolism and excretion of a barbiturate will be impaired, and therefore hypnosis will be prolonged as compared to that of a control group. The inhibitors of the hepatic mixed function peroxidase enzymes act to inhibit CCl₄ activation and its transformation in the trichloromethyl radical. According to Gilani et al., the duration of the pentobarbital-induced sleeping time in animals is considered a good indicator of the activity of microsomal enzymes of the hepatic mixed function peroxidase system. The products resulting from pentobarbital biotransformation by this system are inactive, and thus any drug capable of inhibiting these enzymes activity should increase barbiturate-induced sleeping time. At the doses tested, by intraperitoneal and oral routes, QF did not increase the sleeping time, indicating that it did not alter the activity of the mixed function oxidase enzymes.

Lin et al. demonstrated that two fractions from Ban-zhlian, a Taiwan folk medicine derived from Scutellaria rivularis Benth., exhibited protective effects against CCl₄-induced acute hepatotoxicity in rats. Since this experimental model depends upon the cytochrome P-450 system to produce reactive metabolites, the authors conclude that the fraction’s mechanism of action may be due to the following factors: 1) inhibition of cytochrome P-450 activity; 2) prevention of the process of lipid peroxidation; 3) stabilization of the hepatocellular membrane and 4) enhancement of protein synthesis. Moreover, silymarin has been reported to be capable of modifying several indicators of chronic liver damage induced by CCl₄ in rats.

In conclusion, the quinone fraction from Auxemma oncocalyx, represented mainly by oncocalyxone A, showed a hepatoprotective effect in the models of CCl₄-induced hepatotoxicity in rats and of prolongation of pentobarbital sleeping time in mice, probably due to its antioxidant activity.

Acknowledgements This work had financial support from the Brazilian National Research Council (CNPq).

REFERENCES AND NOTES