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Hepatoprotective activity of *Trichilia roka* on carbon tetrachloride-induced liver damage in rats

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Abstract

Trichilia roka Chiov. (Meliaceae) is a tree widely distributed in tropical Africa. It has been used in Mali folk medicine for the treatment of various illnesses. A decoction of the roots is taken as a remedy for colds and pneumonia, and it is used as a diuretic and in hepatic disorders. We have evaluated the hepatoprotective effects of a decoction of *Trichilia roka* root on CCl_4-induced acute liver damage in rats. Treatment with the decoction showed a significant protective action made evident by its effect on the levels of glutamate oxalacetate transaminase and glutamate pyruvate transaminase in the serum, on the protein content and lipid peroxidation levels in the liver homogenate. Histopathological changes produced by CCl_4, such as necrosis, fatty change, ballooning degeneration and inflammatory infiltration of lymphocytes around the central veins, were clearly recovered by the treatment with *Trichilia* root decoction. On fractionating this extract into diethyl ether-soluble and water-soluble fractions, the activity was retained in the diethyl ether-soluble fraction. Moreover, the administration of decoction prevented a preferential deposition of collagen around the sinusoidal cell layer, which is responsible for the perisinusoidal fibrosis in the early stage of CCl_4 damage. This study showed that treatment with *Trichilia roka* extracts or silymarin (as reference) appeared to enhance the recovery from CCl_4-induced hepatotoxicity. The hepatoprotective properties of *Trichilia roka* may be correlated to polyphenol content of the decoction and its diethyl ether-soluble fraction.

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Introduction

In Africa it is believed that up to 80 % of people consult traditional healers (McGaw et al 1997). A scientific evaluation of plants used by traditional healers is essential before traditional medicine can be incorporated into Africa's official health care system. Many indigenous plants are used for the treatment of liver disorders (Kerharo & Adam 1974). *Trichilia roka* Chiov. (Meliaceae), commonly known as "Sulafinzan" in the Bambara language, is one of the plants used for liver ailments in Mali folk medicine. A decoction of the root is taken in hepatic disorders and as a remedy for colds, pneumonia and as a diuretic (Kokwaro 1976; Malgras 1992).

The plant is also used as a purgative, an antiepileptic, an antipyretic, as a general tonic and for bronchial inflammation (Iwu 1993).

Previous studies have led to the isolation of a number of limonoids (Nakatani et al 1981, 1985), some of which have a wide range of biological activities including insect antifeedant and growth regulation properties, antifungal, bactericidal and antiviral activities (Champagne et al 1992).

McGaw et al (1997) reported that an extract of *T. roka* leaves had inhibitory

activity against cyclooxygenase; moreover, Gunatilaka (1998) showed selective toxicity to DNA repair-deficient yeast of some constituents of *T. roka* stem bark.

There are no pharmacological data available to substantiate the therapeutic value of *T. roka* in liver disorders. Therefore, in this study the hepatoprotective effect of the aqueous extract of *T. roka* root and its diethyl ether-soluble fraction were evaluated on CCl_4-induced acute liver damage in the rat. The results provided evidence to support the traditional usage of *T. roka* for the treatment of liver disease.

Materials and Methods

Experimental animals

Male Wistar rats (180–200 g) were used for all experiments. Rats were maintained under a 12-h light/dark cycle in a temperature and humidity controlled room, with free access to food and water for two weeks before treatment.

Preparation of plant extracts

T. roka fresh root was collected in the belt of Bamako (Mali). Plant identity was confirmed by comparison with the authentic sample of *T. roka* preserved in the herbarium of the Division of Traditional Medicine of Bamako.

Plant material was air dried and powdered. A 10% decoction was prepared and after filtration, the liquid was lyophilized (yield 13.64 g).

A quantitative estimate of the polyphenol content in the decoction was carried out according to the method in the *European Pharmacopoeia* (1997). The assay consisted of quantitating (by colorimetry after reaction with phosphotungstic acid, relative to pyrogallol as a standard) the total phenols present in the extract, before and after precipitation with hide powder.

A portion of lyophilized decoction (10 g) was redissolved in water (100 mL) and then extracted with diethyl ether (100 mL × 2). The organic and aqueous extracts obtained were dried under reduced pressure. The yields were 0.075 g for the organic extract and 9.05 g for the aqueous extract. The composition of *T. roka* extracts was characterized by thin-layer chromatography. For TLC analysis, silica gel 60 plates (Merck) 20 × 20 cm were developed with chloroform–ethyl acetate–formic acid (5:4:1). Finally, the plates were dried and sprayed with 5% methanolic phosphomolybdic acid (Randerath 1965).

Moreover, the presence of limonoids was tested redissolving a few milligrams of the organic extract in diethyl ether (5 mL) and then adding the same volume of hexane to give a precipitate (Nakatani et al 1994).

Treatment of animals

Liver damage was induced in rats by a single intraperitoneal injection of CCl_4 (1.0 mL kg^{-1} in a 50% v/v olive oil solution) (Ohta et al 1998). The hepatoprotective effect of *T. roka* was evaluated by administration of different extracts (5 mL kg^{-1}, p.o., in a 1% w/v carmellose solution) at varying doses, expressed as g of dried starting materials, 2 h after CCl_4 challenge. Silymarin was used as the reference (5 mL kg^{-1}, p.o., in a 1% w/v carmellose solution).

The control groups were treated intraperitoneally with an olive oil solution (1 mL kg^{-1}) and 2 h later received the vehicle (5 mL kg^{-1}, p.o., in a 1% w/v carmellose solution).

All animals were killed by cervical dislocation under ether anaesthesia 24 h after CCl_4 administration. Blood samples were collected and livers were removed. Liver sections were taken from each lobe for histopathological observation; the remaining livers were stored at –80°C before use.

Assessment of liver function

The blood samples were left to coagulate at room temperature and then serum was separated by centrifugation at 2500 rev min^{-1} for 20 min. Serum levels of glutamate oxalacetate transaminase and glutamate pyruvate transaminase were measured using UV-Autom test kits (Sentinel CH Milano, Italy). The data were reported in IU L^{-1}.

The frozen liver samples were homogenized in ice-cold 1.15% w/v potassium chloride to make a 10% w/v liver homogenate. The protein content was determined by the method of Bradford (1976).

The level of lipid peroxides was estimated as malondialdehyde (μmol (g tissue)$^{-1}$) by the measurement of thiobarbituric acid (TBA)-reactive substances at 535 nm (Shimadzu UV-1601 spectrophotometer) as described by Buege & Aust (1978).

Histological observation

Liver sections were fixed in 4% *p*-formaldehyde and washed in phosphate buffer 0.2 M pH 7.4 at 4°C for 12 h. After dehydration, the tissue was embedded in paraffin, cut into 5-μm sections, stained with the haematoxylin–eosin dye and, finally, observed under a photomicroscope (Olympus BH_2).

Morphological changes examined in different liver sections were classified using six categories: necrosis, ballooning of hepatocytes, swelling of hepatocytes, inflammatory cell infiltration, presence of lipid droplets, and normal hepatocytes (Plaa & Charbonneau 1994).

The presence of hepatic collagen was evaluated by staining liver sections with Azan-Mallory dye.

Statistical analysis

Values were given as arithmetic mean ± standard error. Student's *t*-test was used to compare unpaired means of two data sets.

Results

The polyphenol content of *T. roka* root decoction was 9.6% of the dried residue. TLC analysis confirmed the presence of polyphenolic compounds in *T. roka* extracts. There was evidence of limonoids in the organic extract.

Biochemical examination

The effects of *T. roka* on CCl_4-liver injury are summarized in Table 1. Different extracts of varying doses (lyophilized decoction 0.25, 0.50 and 1.0 g kg^{-1}; organic extract, 0.5 and 1.0 g kg^{-1}; aqueous extract, 1.0 g kg^{-1}; silymarin, 0.05 and 0.10 g kg^{-1}) were tested to evaluate the hepatoprotective activity.

The results indicated that 24 h after a single injection of CCl_4 a marked increase in liver transaminase activity and lipid peroxide levels were provoked, as well as a decrease in protein content, which were significantly different from those observed in the control group. When the rats were administered lyophilized decoction (1.0 g kg^{-1}), organic extract (1.0 g kg^{-1}) or silymarin (0.10 g kg^{-1}), the elevated enzymatic activities caused by CCl_4 intoxication were reduced.

Administration of lyophilized decoction at 0.25, 0.50 or 1.0 g kg^{-1} significantly inhibited ($P < 0.01$) the CCl_4-mediated increase in lipid peroxides, while the hepatic protein content was increased ($P < 0.05$). Treatment with 1.0 g kg^{-1} organic extract produced a significant decrease in the formation of TBA (thiobarbituric acid)-reactive substances. Administration of the reference substance silymarin 0.10 g kg^{-1} determined an increment of hepatic protein content, which was higher than the control, and a reduction in the level of lipid peroxides. The lower doses of the same extracts were without significant activity. The aqueous extract did not afford any hepatoprotective properties.

Histological observation

Figure 1 shows photomicrographs of haematoxylin-eosin stained liver tissue. Treatment with CCl_4 caused severe damage showing fatty change, massive necrosis, lipid droplets, and broad infiltration of lymphocytes

Table 1 Effects of *Trichilia roka* extracts on CCl_4-induced hepatotoxicity in rats.

Groups	Glutamate oxalacetate transaminase (IU L^{-1})	Glutamate pyruvate transaminase (IU L^{-1})	Protein (mg (g tissue)$^{-1}$)	Malondialdehyde (μmol (g tissue)$^{-1}$)
Control	154.30 ± 25.30	41.81 ± 2.89	22.62 ± 0.079	0.75 ± 0.050
CCl_4	657.19 ± 23.18†	328.30 ± 63.65†	14.43 ± 1.21†	1.05 ± 0.030†
CCl_4 + *Trichilia roka* organic extract (0.5 g kg^{-1})	790.50 ± 21.11	478.60 ± 65.53	15.57 ± 0.40	0.94 ± 0.044
CCl_4 + *Trichilia roka* organic extract (1g kg^{-1})	338.49 ± 55.36	180.72 ± 48.75	16.92 ± 1.23	0.83 ± 0.020**
CCl_4 + *Trichilia roka* aqueous extract (1g kg^{-1})	540.73 ± 64.13	315.40 ± 53.85	18.23 ± 0.45	0.82 ± 0.089
CCl_4 + *Trichilia roka* lyophilized decoction (0.25 g kg^{-1})	960.20 ± 49.27	490.85 ± 76.81	17.98 ± 0.39*	0.75 ± 0.031**
CCl_4 + *Trichilia roka* lyophilized decoction (0.5 g kg^{-1})	571.09 ± 43.00	262.20 ± 31.38	17.56 ± 0.47*	0.64 ± 0.047**
CCl_4 + *Trichilia roka* lyophilized decoction (1g kg^{-1})	317.36 ± 77.64*	230.04 ± 69.71	20.06 ± 0.65*	0.71 ± 0.043**
CCl_4 + silymarin 0.05 g kg^{-1}	745.52 ± 140.21	389.17 ± 62.01	18.92 ± 0.51	0.96 ± 0.098
CCl_4 + silymarin 0.10 g kg^{-1}	332.43 ± 16.60*	191.57 ± 20.88	25.70 ± 0.89**	0.62 ± 0.049**

The results are expressed as mean ± s.e. of six rats/group. Student's *t*-test: †$P < 0.05$ compared with controls; *$P < 0.05$, **$P < 0.01$ compared with CCl_4-treated groups.

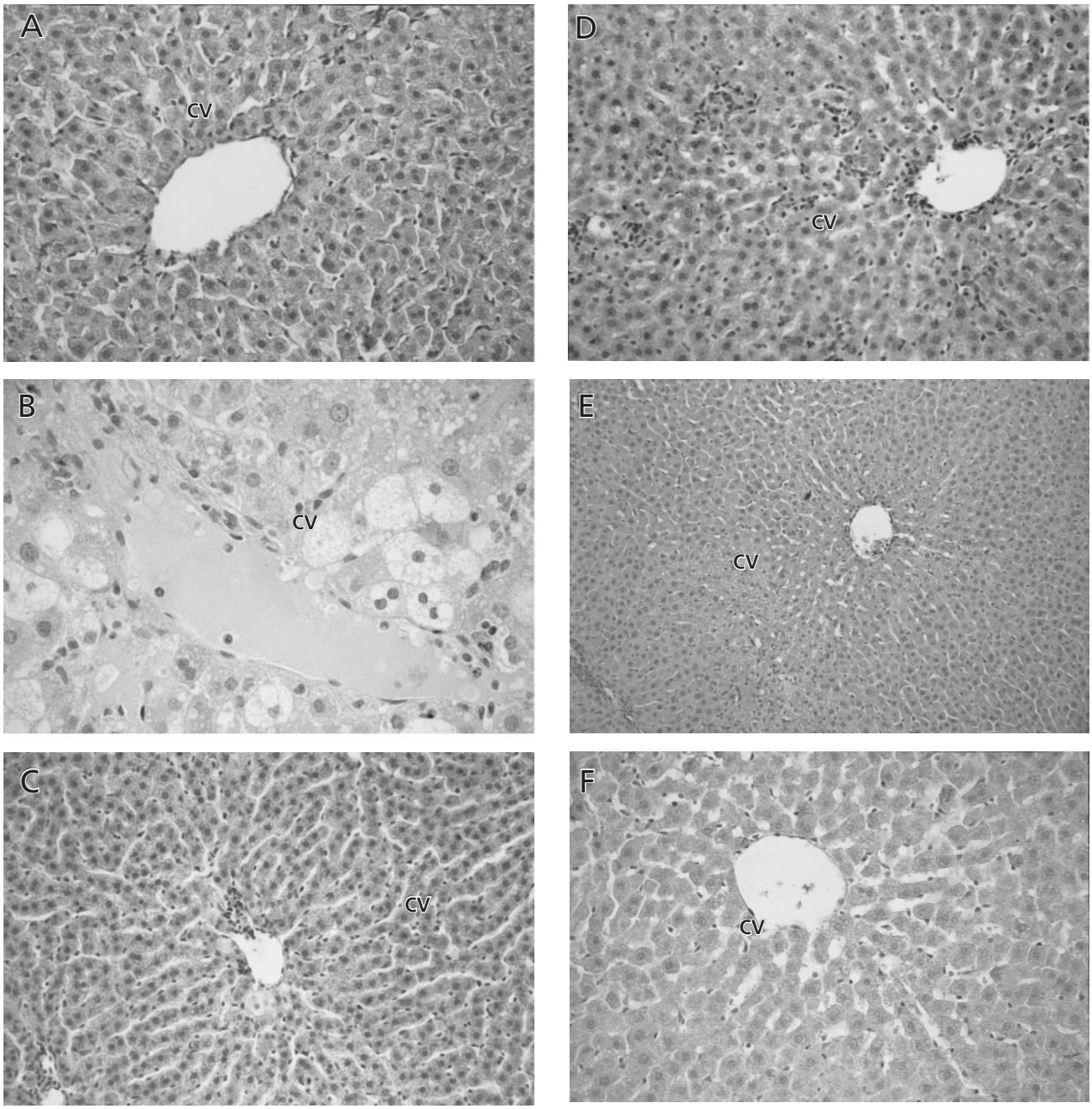

Figure 1 Photomicrographs of liver sections (haematoxylin–eosin). A. Control group, × 200; B. CCl_4 (1.0 mL kg^{-1} in 50% v/v olive oil, i.p.), × 400; C. CCl_4 + *Trichilia roka* decoction (1.0 g kg^{-1}, p.o.), × 200; D. CCl_4 + *Trichilia roka* decoction (0.5 g kg^{-1}, p.o.), × 200; E. CCl_4 + organic extract (1.0 g kg^{-1}, p.o.), × 100; F. CCl_4 + silymarin (0.10 g kg^{-1}, p.o.), × 200. cv, central vein.

around the central vein as compared with the control group (Figure 1A, B). Less damage was present in the livers of the rats treated with *T. roka* extracts.

The results indicated that the decoction at the dose of 1.0 g kg^{-1} showed the best hepatoprotective activity (Figure 1C). This effect was similar to that produced by 0.10 g kg^{-1} silymarin (Figure 1F). Administration of 0.5 g kg^{-1} decoction or 1.0 g kg^{-1} organic extract produced less pronounced hepatoprotective effect (Figure 1D, E).

Figure 2 shows photomicrographs of liver sections stained with the Azan-Mallory dye. CCl_4 treatment

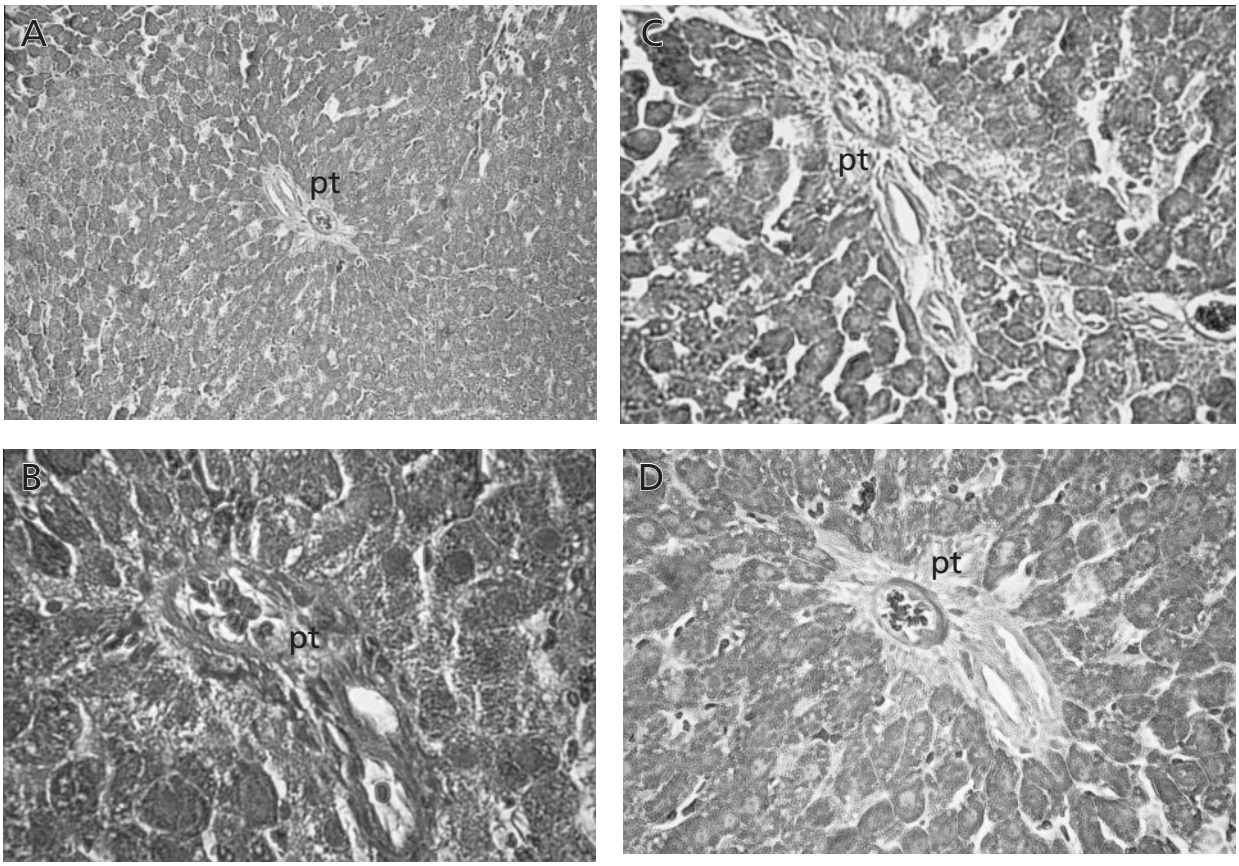

Figure 2 Photomicrographs of liver sections (Azan-Mallory). A. Control group, × 100; B. CCl_4 (1.0 mL kg^{-1} in 50 % v/v olive oil, i.p.), × 400; C. CCl_4 + *Trichilia roka* decoction (1.0 g kg^{-1}, p.o.), × 400; D. CCl_4 + silymarin (0.10 g kg^{-1}, p.o.), × 400. pt, portal tract.

induced a marked increase in collagen fibre deposition. Many bundles of collagen fibre were deposited, especially in the portal tract branching in the perisinusoidal space and surrounding the hepatocytes as compared with the control group (Figure 2A, B). Treatment with *T. roka* decoction at the highest dose (1.0 g kg^{-1}) showed an evident hepatoprotective effect. In fact, the deposition of collagen fibres in the portal tract was reduced and comparable with that observed in the group treated with silymarin (Figure 2C, D).

Discussion

Considering all the results, we can confirm that *T. roka* exerted a clear protective action against CCl_4-induced hepatic damage. This action was predominantly due to administration of decoction and its diethyl ether-soluble fraction, both at the highest dose (1.0 g kg^{-1}). It has been shown that these extracts exert their action preserving the structural integrity of the hepatocellular membrane as evident from the protection provided as compared with the enzymatic levels in CCl_4-treated rats.

Trichilia extracts afforded protection through decreased production of free radical derivatives as evident from malondialdehyde levels. By decreasing lipid peroxidation it has been hypothesized that the plant extracts may be good inhibitors of chemically-induced oxidative stress. This could be explained, at least in part, by the type of compounds present in the extracts. The decoction contained different polyphenolic-like compounds. Polyphenols were also present in the organic and in the aqueous extracts, even if the last one (1 g kg^{-1}) failed to protect against CCl_4-induced liver damage. So, it has been hypothesized that the active principles responsible for the hepatoprotective activity were those soluble in diethyl ether. Recently, intense speculation has been generated in relation to the possible role which simple plant phenols and polyphenols may have in the treatment of various diseases which are associated with the

presence of pro-oxidant status, for their ability to scavenge reactive oxygen species in cellular pro-oxidant states (Haslam 1996).

The contribution of polyphenolic compounds may also justify the morphological results. Treatment with *T. roka* extracts prevented the intralobular bundles of collagen fibres and this facilitated the metabolic exchange between hepatocytes and blood. The hepatic free proline pool size is implicated in the regulation of collagen synthesis (Chvapil & Ryan 1973) and it is known that polyphenols bind most strongly to extended proteins with a high proline content (Hagerman & Butler 1981). Collagen falls in the category of proline rich protein.

The interaction of plant polyphenols with the proline rich protein collagen in the liver may prevent a preferential deposition of collagen around the sinusoidal cell layer which is also responsible for the perisinusoidal fibrosis in an early stage of CCl_4 damage. Our data suggested that *T. roka* could be a promising antifibrotic agent. Findings supporting this view are the comparative potencies of *T. roka* decoction and silymarin treatment. The antifibrogenic properties of silymarin were documented by a variety of parameters including histological data (Fuchs et al 1997). However, further studies are needed to elucidate the underlying mechanism of collagen degradation and to confirm the active principles responsible for the hepatoprotective effect. In fact, the involvement of limonoids, which are present in the diethyl ether extract, could be hypothesized and their possible role in the activity of the detoxifying enzyme glutathione-S-transferase (Bagge 1998).

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