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Research report

Ethyl-acetate fraction of *Trichilia catigua* restores long-term retrograde memory and reduces oxidative stress and inflammation after global cerebral ischemia in rats

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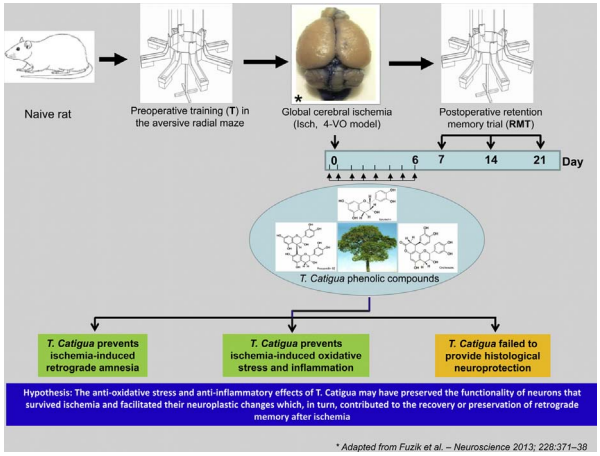
GRAPHICAL ABSTRACT



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ABSTRACT

We originally reported that an ethyl-acetate fraction (EAF) of *Trichilia catigua* prevented the impairment of water maze learning and hippocampal neurodegeneration after transient global cerebral (TGCI) in mice. We extended that previous study by evaluating whether *T. catigua* (*i*) prevents the loss of long-term retrograde memory assessed in the aversive radial maze (AvRM), (*ii*) confers hippocampal and cortical neuroprotection, and (*iii*) mitigates oxidative stress and neuroinflammation in rats that are subjected to the four vessel occlusion (4-VO) model of TGCI. In the first experiment, naive rats were trained in the AvRM and then subjected to TGCI. The EAF

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was administered orally 30 min before and 1 h after TGCI, and administration continued once per day for 7 days post-ischemia. In the second experiment, the EAF was administered 30 min before and 1 h after TGCI, and protein carbonylation and myeloperoxidase (MPO) activity were assayed 24 h and 5 days later, respectively. Retrograde memory performance was assessed 8, 15, and 21 days post-ischemia. Ischemia caused persistent retrograde amnesia, and this effect was prevented by *T. catigua*. This memory protection (or preservation) persisted even after the treatment was discontinued, despite the absence of histological neuroprotection. Protein carbonyl group content and MPO activity increased around 43% and 100%, respectively, after TGCI, which were abolished by the EAF of *T. catigua*. The administration of EAF did not coincide with the days of memory testing. The data indicate that antioxidant and/or antiinflammatory actions in the early phase of ischemia/reperfusion contribute to the long-term antiamnesic effect of *T. catigua*.

1. Introduction

Ischemic brain disease is the third largest cause of mortality and one of the most frequent causes of disability, with a high social and economic burden. Transient global cerebral ischemia (TGCI) is a rapidly developing disorder that is usually caused by reversible cardiac arrest [51]. This event interrupts blood flow in the brain as a whole. The severity of long-term functional deficits is tightly correlated with lesions in the hippocampus, the thalamus, and various cortical regions [30]. Patients who survive cardiac arrest or other less frequent causes of global brain ischemia or hypoxia commonly develop cognitive deficits and executive dysfunction, including anterograde and/or retrograde amnesia, attention deficits, verbal communication disability, spatial/temporal disorientation, and decision-making impairments [2].

Many compounds have been shown to have neuroprotective properties in animal models of cerebral ischemia/reperfusion (I/R). However, no clinically effective therapies are available to treat functional outcomes associated with I/R. Despite the many factors that contribute to this translational dilemma, the identification of new substances that are safe and effective is imperative [16,22].

Oxidative stress plays a major role in the complex cascade of neuropathological changes that are triggered by ischemia. Under conditions of prooxidation/antioxidation homeostasis, oxidative stress implies an imbalance toward prooxidation, with an increase in the generation of reactive oxygen species (ROS) and/or depletion of endogenous antioxidant substances. The overproduction and accumulation of ROS can damage all types of biological molecules, including proteins, lipids, and DNA, which can have serious deleterious consequences. Among these molecules, proteins may be the most immediate and vulnerable targets of oxidative damage in cells [21]. Together with oxidative stress, inflammation can be detrimental to brain tissue. Global cerebral ischemia induces a persistent inflammatory response that involves polymorphonuclear leukocytes (or granulocytes), T-cells, and at a later stage macrophages and microglial cells that secrete proinflammatory mediators, such as interleukin-1 (IL-1), IL-6, IL-10, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ), which are all potentially cytotoxic [34,52,54]. This inflammatory response has been shown to persist for up to 1 year after I/R [52]. Under conditions of global cerebral ischemia, oxidative stress [32,53] and neuroinflammation [52] peak within the first 24–48 h of reperfusion, which may provide a window of opportunity for pharmacological intervention. Substances with antioxidant and antiinflammatory properties may have therapeutic efficacy in alleviating the outcomes of cerebral ischemia [16].

Trichilia catigua (Meliaceae) is a medium-sized tree that is distributed in various South American countries. In Brazil, it is known as “catuaba,” and its commercial preparations have been used in folk medicine as physical and mental tonics, especially as a sexual stimulant [43]. Epicatechins, procyanidins (B2, B4, and C1), and cinchonins (Ia, Ib, IIa, and IIb) have been identified as major components of the crude extract of the stem bark of *T. catigua* [48]. Extracts that were obtained from *T. catigua* have been shown to exert antioxidant [31,56], antiinflammatory [10], antimicrobial, and antiviral [43] activities in *in vitro* studies. This pharmacological profile of *T. catigua* is similar to (−)-epigallocatechin-3-gallate, the main constituent and likely the most active compound of green tea (*Camelia sinensis*) [4]. In *in vivo* behavioral models, treatment with an extract of *T. catigua* exerted antinociceptive [45], antidepressant [13,17], and memory-enhancing [17] effects. Compared with ascorbic acid and the vitamin E analog Trolox, all phenolic compounds that have been isolated from *T. catigua* had antioxidant activity in the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging test [17,48,56].

To our knowledge, only two studies have reported the effects of *T. catigua* in animal models of cerebral ischemia. In one study, brain ischemia was induced by *in vitro* oxygen/glucose suppression. Under such conditions, the addition of an hydroethanolic extract of *T. catigua* to the incubation medium reduced the levels of ROS and lactate dehydrogenase and improved mitochondrial viability [31]. The other study from our laboratory used the bilateral common carotid artery occlusion (BCCAO) model of TGCI in mice. Treatment with a semi-purified EAF from the crude extract of *T. catigua* reduced learning deficits in the water maze test and prevented pyramidal cell death in the hippocampus [57]. These two studies suggest that *T. catigua* may be a source of substances with neuroprotective properties. Additional studies are needed, however, to advance our understanding of the neuroprotective properties of *T. catigua* under conditions of cerebral I/R.

To extend our previous study in mice [57], the present study evaluated rats in the aversive radial maze (AvRM) to investigate whether the EAF of *T. catigua* (*i*) protects against the ischemia-induced deterioration of remote retrograde memory, (*ii*) prevents hippocampal and cortical neurodegeneration, and (*iii*) prevents oxidative stress and inflammation after TGCI.

2. Material and methods

2.1. Plant material and extract preparation

The bark of *T. catigua* was acquired in Caetité, Bahia, Brazil, in May 2011. A voucher specimen was identified by Dr. Cássia Mônica Sakuragui, Universidade Federal do Rio de Janeiro, RJ, Brazil, and deposited at the Herbarium of State University of Maringá (HUEM no. 19.434), Maringá, PR, Brazil. The extracts were obtained as described previously [17,48]. Briefly, the crude extract (CE) was produced with acetone-water (7:3, v/v) in a drug:solvent proportion of 10%. The EAF of *T. catigua* was then obtained after partition with ethyl acetate.

2.2. Chromatographic evaluation

The EAF of *T. catigua* was evaluated by high-performance liquid chromatography (HPLC) according to Longhini et al. [35]. We used a Thermo HPLC device that was equipped with a photodiode array (PDA) spectrophotometric detector module (Model Finnigan Surveyor PDA Paus Detector), controller software (Chromquest), autosampler (Finnigan Surveyor Autosampler Plus) with a 10 µl loop (total injection), integral pumps, and degasser (Finnigan Surveyor LC Pump Plus). The