Involvement of Serotonin in the Antidepressant-like Effect of Extract from Kielmeyera coriacea Stems

Yara C.F. Goulart1, Juliana V.C. Martins1, Adair R. Santos2, Leandro Y. Moreira1, João Batista Calixto2, Diógenes A.G. Cortez1, and Elisabeth A. Audi1

1Department of Pharmacy and Pharmacology, University of Maringá, PR, Brazil; 2Department of Pharmacology, Federal University of Santa Catarina, SC, Brazil

Abstract

The stems of Kielmeyera coriacea Mart. (Clusiaceae) (syn. “pau-santo”) are a therapeutic herbal in Brazilian folk medicine. This study investigates the effects of a hydroethanolic extract (HE) from Kielmeyera coriacea stems on the central nervous system (CNS) of rats. Chronic administration by gavage of the extract (60.0 mg/kg) revealed decreased immobility time in the forced swimming test (FST). This effect of the extract was compared with chronic treatment by gavage of fluoxetine (10.0 mg/kg) and nortriptyline (15.0 mg/kg). The antidepressant-like effect of the HE from Kielmeyera coriacea in the FST was also investigated in association with the intra-dorsal raphe nucleus microinjection of 5-hydroxytryptamine (serotonin; 5-HT) or R(+)-8-hydroxyl-2-(di-n-propylamino)tetralin (8-OHDPAT), a 5-HT1A specific agonist receptor. The ligands, 5-HT (5.0 nmol) and 8-OHDPAT (0.6 and 1.0 nmol), significantly increased immobility time per se and blocked the antidepressant-like effect of the extract. Biochemical investigations employing an in vitro synaptosomal assay showed uptake inhibition by the extract of [3H]5-HT, [3H]noradrenaline (NA), and [3H]dopamine (DA) in the rat brain. These results suggest that serotonergic neurotransmission is involved in the antidepressant-like activity of the extract, as shown by the interaction with microinjected 5-HT and 8-OHDPAT, and that 5-HT, NA, and DA uptake inhibition may contribute to this effect.

Keywords: Antidepressant activity, dorsal raphe nucleus, forced swimming test, Kielmeyera coriacea, open-field test, rat, serotonin uptake.

Introduction

Kielmeyera coriacea Mart. is a tree of the Clusiaceae family and popularly known in Brazil as “pau santo.” A decoction of the stems is used to treat various tropical diseases including schistosomiasis, leishmaniasis, malaria, and fungal and bacterial infections, among others (Alves et al., 2000). Previous analysis of the extracts from Kielmeyera coriacea by high-performance liquid chromatography (HPLC) with photodiode array detection (LC-UV) showed the presence of xanthones, triterpenes, and biphenyl derivatives, and antifungal activity against Cladosporium cucumerinum and Candida albicans was exhibited. Combined with HPLC using a thermospray mass spectrometry interface (TSP/LC-MS), it was possible to identify the xanthones 2-hydroxy-1-methoxyxanthone, 3-hydroxy-2,4-dimethoxyxanthone, 4-hydroxy-2,3-dimethoxyxanthone, swertinin, 6-hydroxy-1,3,5-trimethoxyxanthone, 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone, and kielcorin (Bennet & Lee, 1988; Cortez et al., 1998).

Hypericum perforatum L., a plant from the same family (Clusiaceae), is considered an effective alternative in the treatment of mild to moderate depression (Josey & Tackett, 1999; Wheatly, 1999). Active extracts of Hypericum perforatum have been shown to inhibit the synaptosomal uptake of dopamine (DA), noradrenaline (NA), and serotonin (5-HT) (Muller et al., 1997; Muruganandam et al., 2000).
Materials and Methods

Plant material

*Kielmeyera coriacea* was collected near Mogi-Guaçu (São Paulo, Brazil) in July 1999. A voucher specimen (no. SP298463) was deposited with the herbarium of the State Botanical Institute, São Paulo, Brazil. Species identification was performed by Dr. Maria Claudia Young of the same institution.

Plant extract

The dried and crushed stems (1.0 kg) of *Kielmeyera coriacea* were exhaustively extracted with 38 L of ethanol/water (9:1) at room temperature for 7 days, yielding 167.3 g of extract after evaporation of the solvents and lyophilization. The resulting compound was registered by the University of Maringá under patent application no. 001342 with the National Patents Institute (INPI) on October 9, 2002.

Animals

Male Wistar rats aged 55–63 days, weighing 240–270 g (Central Biotério, University of Maringá) were used. The rats were housed in groups of four per cage and maintained on a 12h light:dark cycle (lights on at 0700 h) under controlled temperature (22 ± 1°C), with food and water freely available. The animals were maintained in this situation for 3 days before the surgery for acclimatization and during the postsurgery period. All experiments were carried out between 0800 and 1200 h. The experimental procedures adopted were approved by the UEM Ethics Committee (084-02/COBEA) and follow the norms recommended as international guiding principles for Biomedical Research Involving Animals (CIMS, Geneva, 1985).

Treatment schedule

Fluoxetine hydrochloride (SP Pharma), nortriptyline hydrochloride (Cristália Produtos Químicos Farmacêuticos Ltda.), and HE from *Kielmeyera coriacea* were dissolved in saline (0.9% NaCl). Solutions of 8-OHDPAT hydrobromide (Research Biochemical Incorporated, St. Albans, UK) and serotonin creatinine sulfate complex (RBI) were dissolved in saline plus 1% ascorbic acid (vehicle). HE from *Kielmeyera coriacea* or saline were chronically administered by gavage once a day for 45 days and 1 h before testing. The 5-HT ligands or vehicle were microinjected intra-DRN 10 min before the FST or the OFT. Drug doses were based on pilot studies. The antidepressant drugs, nortriptyline and fluoxetine, were administered by intraperitoneal (i.p.) route and used as positive control to standardize the FST.

Surgery

Rats were anesthetized with tribromoethanol (2.5%, 10.0 mL/kg) and positioned in a stereotaxic frame device (Koft Instruments, Tujunga, CA, USA). The skull was exposed, and the incisor bar was adjusted such that bregma and lambda were positioned at the same height. Two perforations were made in the skull to accommodate screws, which, together with dental cement, held the cannulae in place. The stereotaxic coordinates from the Paxinos & Watson atlas (1986) with reference to bregma for the tip of the 15-mm-long stainless-steel cannula (0.6 mm external diameter) rested 0.2 mm above the DRN were AP = −7.1 mm, L = 4.0 mm, DV = 5.3 mm. The cannula was inserted into the DRN

The central 5-hydroxytryptamine (5-HT) system plays an important role in the etiology and treatment of anxiety and mood disorders. Ascending 5-HT pathways originating from dorsal raphe nucleus (DRN) have received attention so far for controlling aversion, modulating through different subtypes of receptors the neural substrates of anxiety, panic, and depression. The DRN located in the midbrain exhibits the highest 5-HT transporter density in both human and rat brains (Artigas et al., 1996). Previous studies have shown that the discharge rate of serotonergic neurons is sensitive to drugs that increase the synaptic availability of serotonin in the brain. These drugs produce a compensatory decrease in neuronal activity by activating somatodendritic 5-HT$_1$A autoreceptors on serotonergic neurons located within the midbrain raphe (Barnes & Sharp, 1999).

The forced swimming test (FST) is a behavioral model developed to predict the efficacy of antidepressant drugs and is sensitive to compounds acting on the serotonergic and noradrenergic systems. This test induces immobility when a rodent is placed in a tank of deep water for an extended period, making only minimal movements necessary to keep its head above the water. The development of immobility is facilitated by prior exposure to a swimming pre-test. Antidepressant treatments decrease immobility time in the FST (Porsolt et al., 1977; 1978).

The aim of this work was to investigate whether chronic administration of a hydroethonal extract (HE) from *Kielmeyera coriacea* stems induces an anti-immobility effect in the FST or alters locomotor activity in the open field test (OFT) in rats. To investigate the possible contribution of 5-HT to the antidepressant-like effect of the extract, we associated the systemically delivered extract with 5-HT or $R(+)$-8-hydroxyl-2-(di-n-propylamo) (tetralin) (8-OHDPAT) microinjected into the DRN. Correlative biochemical experiments were performed to evaluate the relationship between the behavioral effect of HE from *Kielmeyera coriacea* and its other effects on 5-HT, NA, and DA synaptosomal uptake levels.
at an angle of 34 degrees to the horizontal plane to avoid penetration of the midline sinus and the brain aqueducts. On test days, the rats were injected using needles constructed from 30-gauge steel tubing that extended 2 mm below the tip of the cannulae into the DRN. Volumes of 0.5 μL for the control, 5-HT or 8-OHDPAT were microinjected over a 30-s interval. The needles were kept in position for a further 30 s to allow drug diffusion.

**Histology**

After behavioral testing, the animals were immediately sacrificed, and their brains removed and fixed in 10% formaldehyde solution. After fixation, the brains were sectioned into 50-μm-thick slices in the coronal plane using a freeze microtome. The exact injection sites were checked histologically by an independent investigator.

**Forced swimming test**

On the fifth or sixth day after surgery, rats were individually forced to swim in an open cylindrical container (diameter 30 cm, height 60 cm), containing 45 cm of water at 25 ± 1°C. The test employed was essentially similar to that described by Porsolt et al. (1978), except for the water level. In our laboratory, the water level is increased to 45 cm in order to increase the sensitivity of the test. The rats lack a sense of the water’s depth, and their tails do not touch the bottom of the cylinder. This modification in the procedure is consistent with the practice of other authors (Alley & Kulkarni, 1989; Detke & Lucki, 1995) and should be considered the current standard method. Animals were exposed to a pre-test for 15 min, 24 h prior to the 5-min swim test. After 30 s for acclimatization, the 5-min swim session was videotaped for subsequent measurement of the time of immobility by a trained observer. Each animal was considered immobile when it ceased struggling and swimming and remained floating in the water, making only the movements necessary to keep its head above water. After the test the animals were removed from the water, dried by the experimenter, and placed in cages. The same animals used in the FST were individually placed (24 h after) in one corner of an open field (40 × 40 × 40 cm), divided into 25 identical squares, for evaluation of locomotor activity measured by the total number of squares visited. After 30 s for habituation, during a 5-min period, the number of squares visited (four feet placed in the same square) was recorded. Two fluorescent lights provided diffuse overhead illumination (200 lux at the level of the arena).

**Estimation of brain biogenic amine concentrations**

Drug and test-naïve rats were sacrificed by decapitation for the uptake study. The brain was rapidly removed and the cerebral cortices (for [3H]5-HT and [3H]NA uptake) and striatum (for [3H]DA uptake) were dissected and homogenized in 15 mL (10 mL for striatum) of ice-cold sucrose solution (0.32 M), then diluted with 10 mL (5 mL for striatum) of homogenizing medium, pH 7.4. The nuclear fraction was removed by centrifugation for 10 min at 1000 × g, and the supernatant provided the crude synaptosomal pellet (P2). The P2 pellet was resuspended in ice-cold HEPES buffer, and 0.5 mL samples were preincubated for 10 min (5-HT and NA) or for 5 min (DA) at 37°C with or without the HE from *Kielmeyera coriacea* (3–1000 μM), fluoxetine (100 μM), desipramine (100 μM), or cocaine (10 μM). Uptake was initiated by the addition of 0.1 mL [3H]5-HT, [3H]NA, or [3H]DA (2.9, 4.0, or 3.0 μM) to a final volume of 1000 μL per well. The reaction was stopped 4 min later for NA or 10 min later for 5-HT or DA by adding 2 mL of ice-chilled buffer. Samples were immediately filtered through 0.65-μM cellulose ester filters that were washed three-times each with 4 mL of cold buffer solution. The filters were then placed in glass vials containing 4 mL scintillation liquid, and radioactivity was counted using a Packard liquid scintillation counter (Tri-carb 1600-TR). Nonspecific uptake was measured in parallel probes containing unlabeled neurotransmitters (1 μM 5-HT, NA, or DA). Data are given as a percentage of specific uptakes and refer to the specific uptake obtained from the uptake total minus the nonspecific uptake (Whittaker et al., 1964, Nagi & Delgado-Escueta, 1984).

**Statistical analysis**

Data are provided as the mean ± SEM for each group and were analyzed by one-way variance analysis (ANOVA) followed by Dunnett’s test. Differences were considered significant at p ≤ 0.05.

**Results**

All cannula placements were found to lie within the posterior planes of −7.30 to −7.8 mm to bregma (Fig. 1). Cannula placements located within the DRN were
considered in this study. When the injection site was located well below the DRN in the median raphe nucleus, the animals were discarded. No antidepressant effect in the FST or alteration in locomotor activity in the OFT was found after acute administration of HE of *Kielmeyera coriacea* (results not shown).

Figure 2 shows the results of chronic administration (45 days) by gavage of control (saline), HE from *Kielmeyera coriacea* (60.0 mg/kg), nortriptyline (15.0 mg/kg), and fluoxetine (10.0 mg/kg) in the FST (panel A). Dunnett’s test revealed a significant decrease in immobility time with *Kielmeyera coriacea* (p < 0.05), nortriptyline (p < 0.05), and fluoxetine (p < 0.05) compared with the control group (F(3,27) = 4.287; p = 0.01). Panel B shows the same treatments in rats submitted to the OFT. Fluoxetine induced a decrease in locomotor activity, suggestive of sedative effect (p < 0.05) at the same dose that produced anti-immobility effect (F(3,27) = 4.51, p = 0.01).

Figure 3 shows the results of the intra-DRN microinjection of 5-HT (5.0, 10.0, or 20.0 μmol) (panel A), the selective 5-HT<sub>1A</sub> agonist, 8-OHDPAT (0.3, 0.6, or 1.0 μmol) (panel B), or vehicle (panels A and B), after chronic treatment by gavage with saline. 5-HT (5.0 nmol) (F(3,31) = 3.09, p = 0.04) or 8-OHDPAT (0.6 and 1.0 nmol), (F(3,31) = 3.67, p = 0.02) produced a significant (p < 0.05) increase in the immobility time compared with the control group (intra-DRN vehicle + saline by gavage).

Figure 4 shows the results of the intra-DRN microinjection of HE from *Kielmeyera coriacea* (60.0 mg/kg), associated with the intra-DRN microinjection of vehicle induces a significant anti-immobility effect (p < 0.01)
compared with control group. Intra-DRN microinjection of 5-HT (5.0 nmol), F(3,32) = 16.03, p < 0.0001 or 8-OHDPAT at a doses of 0.6 nmol (F(3,30) = 17.02, p < 0.0001) (panel B) or 1.0 nmol (F(3,33) = 24.70, p < 0.0001) (panel C) reverted the anti-immobility effect produced by chronic treatment with HE from Kielmeyera coriacea (panels A, B, and C).

Locomotor activity in the OFT was not significantly increased by any treatment compared with the control rats (results not shown).

**Discussion**

This study shows that chronic administration of HE from Kielmeyera coriacea stems was active in the well-validated FST model of depression in rats. Thus, HE from Kielmeyera coriacea produced a significant, antidepressant-like effect in the FST in a similar magnitude similar to that of fluoxetine and nortriptyline, used as reference drugs. The result in the FST does not reflect a general increase in motor activity due to the HE from Kielmeyera coriacea as the same dose that induced an antidepressant-like effect did not increase ambulatory behavior in the OFT. Chronic administration of fluoxetine significantly reduced locomotion, an effect that has been described in the literature by antidepressant compounds (Porsolt et al., 1977, 1978).
In our study, there was no decrease in immobility time after acute administration of the HE from *Kielmeyera coriacea* stems. Other studies relating to the effectiveness of antidepressant drugs in the FST have demonstrated decrease in the immobility time after chronic treatments (Detke et al., 1995; Harkin et al., 1999).

The increased immobility time in the FST observed in our study with intra-DRN microinjection of (+)-8-OHDPAT or 5-HT has been related to earlier findings indicating that the intra-DRN microinjection of exogenous 5-HT or 5-HT1A agonists reduces the central serotonergic function mediated by activation of the somatodendritic 5-HT1A autoreceptor, producing reduction of 5-HT release in innervated structures in the forebrain. WAY100635 prevents the suppression of cell firing induced by 8-OH-DPAT (Harkin et al., 1999).

The serotonergic system has long been recognized as playing an important role in mood disorders, specifically in the etiology of depression, and drugs that act on the serotonergic system, like the specific 5-HT1A receptor and their function.

The cell bodies of 5-HT-containing neurons are confined primarily to the DRN of the brain stem and project to widespread regions of the brain where the levels of extracellular 5-HT are determined by the electrical activity of these cells (Jacobs & Azmitia, 1992). Somatodendritic autoreceptors of serotonergic neurons in the DRN may be largely 5-HT1A receptors (Barnes & Sharp, 1999). NA and 5-HT may be involved in the mechanism of action of tricyclic antidepressants and probably correct monoamine deficiency by increasing amine availability at postsynaptic receptor sites by inhibiting monoamine uptake (Duman et al., 1997). In addition, classical antidepressant drugs can modify DA activity (Willner, 1983).

The serotonergic system has long been recognized as playing an important role in mood disorders, specifically in the etiology of depression, and drugs that act on the serotonergic system, like the specific 5-HT1A receptor and their function.

The increased immobility time in the FST observed in our study with intra-DRN microinjection of (+)-8-OHDPAT or 5-HT has been related to earlier findings indicating that the intra-DRN microinjection of exogenous 5-HT or 5-HT1A agonists reduces the central serotonergic function mediated by activation of the somatodendritic 5-HT1A autoreceptor, producing reduction of 5-HT release in innervated structures in the forebrain. WAY100635 prevents the suppression of cell firing induced by 8-OH-DPAT (Harkin et al., 1999).

The serotonergic system has long been recognized as playing an important role in mood disorders, specifically in the etiology of depression, and drugs that act on the serotonergic system, like the specific 5-HT1A receptor and their function.

The cell bodies of 5-HT-containing neurons are confined primarily to the DRN of the brain stem and project to widespread regions of the brain where the levels of extracellular 5-HT are determined by the electrical activity of these cells (Jacobs & Azmitia, 1992). Somatodendritic autoreceptors of serotonergic neurons in the DRN may be largely 5-HT1A receptors (Barnes & Sharp, 1999). NA and 5-HT may be involved in the mechanism of action of tricyclic antidepressants and probably correct monoamine deficiency by increasing amine availability at postsynaptic receptor sites by inhibiting monoamine uptake (Duman et al., 1997). In addition, classical antidepressant drugs can modify DA activity (Willner, 1983).

The cell bodies of 5-HT-containing neurons are confined primarily to the DRN of the brain stem and project to widespread regions of the brain where the levels of extracellular 5-HT are determined by the electrical activity of these cells (Jacobs & Azmitia, 1992). Somatodendritic autoreceptors of serotonergic neurons in the DRN may be largely 5-HT1A receptors (Barnes & Sharp, 1999). NA and 5-HT may be involved in the mechanism of action of tricyclic antidepressants and probably correct monoamine deficiency by increasing amine availability at postsynaptic receptor sites by inhibiting monoamine uptake (Duman et al., 1997). In addition, classical antidepressant drugs can modify DA activity (Willner, 1983).

The cell bodies of 5-HT-containing neurons are confined primarily to the DRN of the brain stem and project to widespread regions of the brain where the levels of extracellular 5-HT are determined by the electrical activity of these cells (Jacobs & Azmitia, 1992). Somatodendritic autoreceptors of serotonergic neurons in the DRN may be largely 5-HT1A receptors (Barnes & Sharp, 1999).

The increased immobility time in the FST observed in our study with intra-DRN microinjection of (+)-8-OHDPAT or 5-HT has been related to earlier findings indicating that the intra-DRN microinjection of exogenous 5-HT or 5-HT1A agonists reduces the central serotonergic function mediated by activation of the somatodendritic 5-HT1A autoreceptor, producing reduction of 5-HT release in innervated structures in the forebrain. WAY100635 prevents the suppression of cell firing induced by 8-OH-DPAT (Harkin et al., 1999).

The serotonergic system has long been recognized as playing an important role in mood disorders, specifically in the etiology of depression, and drugs that act on the serotonergic system, like the specific 5-HT1A receptor and their function.

The increased immobility time in the FST observed in our study with intra-DRN microinjection of (+)-8-OHDPAT or 5-HT has been related to earlier findings indicating that the intra-DRN microinjection of exogenous 5-HT or 5-HT1A agonists reduces the central serotonergic function mediated by activation of the somatodendritic 5-HT1A autoreceptor, producing reduction of 5-HT release in innervated structures in the forebrain. WAY100635 prevents the suppression of cell firing induced by 8-OH-DPAT (Harkin et al., 1999).

The increased immobility time in the FST observed in our study with intra-DRN microinjection of (+)-8-OHDPAT or 5-HT has been related to earlier findings indicating that the intra-DRN microinjection of exogenous 5-HT or 5-HT1A agonists reduces the central serotonergic function mediated by activation of the somatodendritic 5-HT1A autoreceptor, producing reduction of 5-HT release in innervated structures in the forebrain. WAY100635 prevents the suppression of cell firing induced by 8-OH-DPAT (Harkin et al., 1999).

The increased immobility time in the FST observed in our study with intra-DRN microinjection of (+)-8-OHDPAT or 5-HT has been related to earlier findings indicating that the intra-DRN microinjection of exogenous 5-HT or 5-HT1A agonists reduces the central serotonergic function mediated by activation of the somatodendritic 5-HT1A autoreceptor, producing reduction of 5-HT release in innervated structures in the forebrain. WAY100635 prevents the suppression of cell firing induced by 8-OH-DPAT (Harkin et al., 1999).

The consistent block of the anti-immobility effect of the HE from *Kielmeyera coriacea* by 5-HT and by 8-OHDPAT suggests that serotonergic mechanism is involved in the antidepressant-like effect of the extract. Yet, the different effects observed after the association with the exogenous 5-HT or the selective 5-HT1A agonist receptor 8-OHDPAT in rats treated with the HE from *Kielmeyera coriacea* suggests that the antidepressant-like effect of the extract was mediated by 5HT1A receptors.

The consistent block of the anti-immobility effect of the HE from *Kielmeyera coriacea* by 5-HT and by 8-OHDPAT suggests that serotonergic mechanism is involved in the antidepressant-like effect of the extract. Yet, the different effects observed after the association with the exogenous 5-HT or the selective 5-HT1A agonist receptor 8-OHDPAT in rats treated with the HE from *Kielmeyera coriacea* suggests that the antidepressant-like effect of the extract was mediated by 5HT1A receptors.

Neurochemical studies have demonstrated the influence of 5-HT1A agonists on the release of various neurotransmitters in limbic regions of the brain. Microdialysis studies have revealed that 8-OHDPAT elevates DA in the striatum (Barnes & Sharp, 1999) and increases dialysate levels of NA in the frontal cortex (De Derwaerde et al., 1998; Ashby & Jr Minabe, 1996; Di Matteo et al., 1998).

When tested *in vitro*, the HE from *Kielmeyera coriacea* stem inhibits the synaptosomal uptake of [3H]5-HT, [3H]NA, and [3H]DA in a concentration-dependent manner. These *in vitro* results reveal a biochemical mechanism similar to that of the tricyclic, antidepressant, or specific 5-HT reuptake inhibitors, despite the different selectivity seen among these compounds.

The current findings suggest that the serotonergic neurotransmission plays a role in antidepressant-like effect of the extract observed in the FST, as shown by its interaction with microinjected 5-HT and 8-OHDPAT. Noradrenergic and dopaminergic neurotransmissions play a role in this effect of the extract, as shown by its uptake inhibition. The specific mechanisms underlying this behavioral action of the HE from *Kielmeyera coriacea* remain to be better resolved. However, as a first step, this extract should be fractioned and further studied as a potential phytotherapeutic product to treat depressive disorders.

**Acknowledgments**

The authors thank Marcos Alberto Trombelli for technical assistance.

**References**


