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Short communication

Assessment of anxiolytic and panicolytic effects of dichloromethane fraction from stems of *Kielmeyera coriacea*

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ABSTRACT

Kielmeyera coriacea Mart. (Calophyllaceae) is known popularly as "Pau Santo". The hydroethanolic extract (HE) of *Kielmeyera coriacea* stems and its semi-pure dichloromethane (DCM) constituent produced an antidepressant-like effect in rats. The purpose of this study was to investigate the effects of repeated administration (21 days) by gavage of the DCM fraction (5, 10 or 15 mg/kg) in rats submitted to the elevated T-maze (ETM), a model of generalized anxiety and panic disorders. The tricyclic antidepressant imipramine (15 mg/kg) was used as a positive control. Rat locomotion was assessed using the open field test (OFT) following each drug treatment. The 2-hydroxy-1-methoxyxanthone (1), acuparin (2), swertinin (3), 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (4) and 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (5) were identified in DCM fraction, and suggest that the xanthone (4) is related with the antidepressant-like profile of this plant. Pharmacological evaluation showed that DCM fraction (10 and 15 mg/kg) decreased the inhibitory avoidance latency from the closed arm and increased the one-way escape latency from the open arm in the ETM, which is indicative of anxiolytic and panicolytic effects, respectively, as occurs with the positive control, imipramine (15 mg/kg), when compared to their control group (vehicle). Locomotor activity was not significantly altered by the different treatments. This study suggests that the DCM fraction from stems of *Kielmeyera coriacea* can be an important therapeutic alternative in the treatment of anxiety disorders, such as generalized anxiety and panic disorders.

Introduction

Anxiety disorders, such as generalized anxiety disorder, panic disorder, obsessive-compulsive disorder and phobias, are the most common psychiatric conditions (Kessler et al. 2005). The high prevalence of psychiatric diseases is associated with occupational limitations, emotional problems (Schonfeld et al. 1997), lower likelihood of satisfaction in family life, in one's sense of overall well-being (Stein and Heimberg 2004) and impaired quality of life (Henning et al. 2007), resulting in high costs to the public health system (Henderson et al. 2005).

Due to the high comorbidity between anxiety and depressive disorders, antidepressants compounds are considered first-line medications in the treatment of these pathologies (Hoffman and Mathew 2008).

Although the available pharmacological treatments for these disorders are effective, they have many limitations, including initial worsening of anxiety symptoms, sleep disturbances, sexual side effects, gastrointestinal problems, weight gain, drug–drug interactions, withdrawal and dependence issues (Baldessarini 2006).

For this reason, and because of their prevalence and the high degree of suffering they cause, anxiety disorders are among the most common reasons for searching for complementary therapies (Eisenberg et al. 1998) and self-medication with medicinal herbs (Astin 1998).

It is estimated that Brazil has the greatest plant biodiversity in the world (Guerra and Nodari 2001), and *Kielmeyera coriacea* Mart. (Calophyllaceae), known as "Pau Santo", is just one of many potentially useful plants available.

The hydroethanolic extract (HE) of *Kielmeyera coriacea* stems (60 mg/kg) and its semi-pure dichloromethane (DCM) constituent (5 mg/kg), but not the extract of *Kielmeyera coriacea* leaves (30–120 mg/kg) (Audi et al. 2002), reduce immobility time in the forced swimming test (FST), without altering locomotor activity in the open-field test (OFT), as occurs with tricyclic antidepressant imipramine (20 mg/kg), when chronically administered (Martins et al. 2004, 2006), suggesting an antidepressant-like profile. The HE

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of *Kielmeyera coriacea* stems inhibits in a concentration-dependent manner the synaptosomal uptake of [^{3}H] serotonin (5-HT), [^{3}H] noradrenaline (NA) and [^{3}H] dopamine (DA) (Goulart et al. 2007), suggesting that these neurotransmitters are involved in the mechanism of action.

The aim of this work was to assess the anxiolytic and panicolytic effects of the DCM fraction on rats subjected to the elevated T-maze (ETM) test.

Materials and methods

Plant material

Kielmeyera coriacea was collected near Mogi Guaçu (São Paulo, Brazil) in December 2007. The analysis and identification of *Kielmeyera coriacea* were carried out by Dr. Maria Claudia Young of the Instituto Botânico de São Paulo. A voucher specimen (#SP 298463) was deposited with the Herbarium of the institute.

Extraction and fractionation

Dried and crushed stems (2 kg) of *Kielmeyera coriacea* were exhaustively extracted with ethanol/water (9:1) at room temperature, yielding 342.1 g of extract after evaporation of the solvents and lyophilization. The lyophilized extract (329.8 g) obtained from *Kielmeyera coriacea* stems was submitted to vacuum-column chromatography on silica gel (70–230 mesh) using step gradient elution with hexane, CH_2Cl_2, AcOEt, acetone and MeOH–H_2O (9:1) to yield five fractions (F1: 3.8 g), (F2: 33.6 g), (F3: 48.6 g), (F4: 39.1 g) and (F5: 198.7 g) after the organic solvent was removed under vacuum at 40 ± 1 °C. The different compounds in DCM fraction from stems of *Kielmeyera coriacea* were identified by comparison with standard samples and by analysis of HPLC–UV.

Behavioral tests

Animals

Male Wistar rats weighing 230–250 g (55 days) were used in this study and housed in groups of four per cage, with food and water freely available. The animals were maintained on a 12-h light:dark cycle (lights on at 07:00 h) under controlled temperature (22 ± 1 °C). All experiments were carried out between 08:00 and 12:00 h. The experimental procedures adopted were approved by the UEM Ethics Committee (049/2010-CEEA), and followed the norms recommended as international guiding principles for Biomedical Research Involving Animals (CIMS), Geneva, 1985.

Drugs

DCM fraction was solubilized in saline (0.9% NaCl) containing 3% Tween 80. Imipramine chlorhydrate was used as positive control (Cristália). The control group was treated with the vehicle (0.9% NaCl plus 3% Tween 80).

Elevated T-maze (ETM) and open field test (OFT)

The tests employed were similar to that described by Roncon et al. (2010).

Treatment

To determine the dose-response curve of DCM fraction, the animals were treated for 21 days with DCM (5, 10 and 15 mg/kg, $n = 10$–15) or vehicle ($n = 12$) by gavage (i.g.) route or imipramine (15 mg/kg, $n = 10$) by intraperitoneal (i.p.) route. On the 20th day, the pre-tests were carried out, in which the animals were confined in one of the open arms of the ETM for 30 min, and after the pre-test received their treatment (drugs or vehicle). On the 21st day of treatment, the animals were treated (drugs or vehicle) and submitted to the behavioral tests 60 min after the treatment.

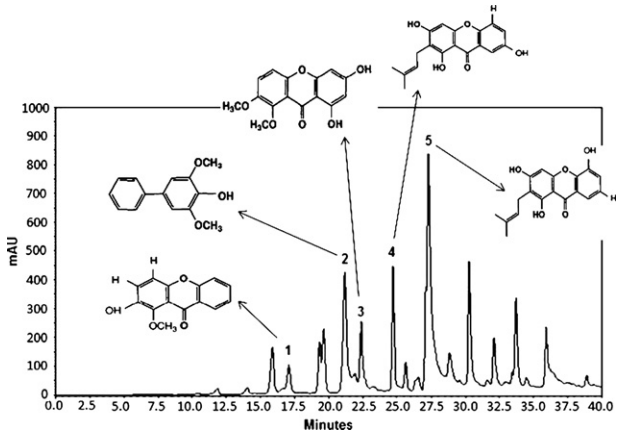

Fig. 1. High performance liquid chromatography chromatogram of DCM fraction from stem of *Kielmeyera coriacea*. Conditions: Phenomenex ODS column; step gradient of acetonitrile–water (containing 0.05% trifluoroacetic acid): 30 → 100% CH_3CN over 40 min; flow rate 0.6 ml/min; temperature: 40 °C; detection UV 254 nm. 2-Hydroxy-1-methoxyxanthone (1); aucuparin (2); swertinin (3); 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (4); 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (5).

Statistical analysis

Repeated measure analyses of variance (RMANOVA) were used to analyze both avoidance and escape data. The treatments were considered as the independent factors, and the tests (baseline, avoidance 1–2 and escape 1–3) as the repeated measures. When appropriate, one-way ANOVA followed by Duncan's *post hoc* multiple comparison test were used. Locomotion data were analyzed by one-way ANOVA followed by Duncan's *post hoc* multiple comparison test. Differences between groups were considered significant if $p \leq 0.05$.

Results

The 2-hydroxy-1-methoxyxanthone (1), aucuparin (2), swertinin (3), 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (4) and 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (5) were identified, with retention times of 17.0 min, 21.1 min, 22.3 min, 24.6 min and 27.2 min, respectively (Fig. 1).

Fig. 2 shows the effects of repeated administration of vehicle (control group), imipramine (15 mg/kg), or DCM (5, 10, or 15 mg/kg) in the ETM and OFT, respectively. The RMANOVA for the inhibitory avoidance test showed a significant main effect on the trials [$F(2,108) = 15.53$; $p < 0.001$], on the treatment [$F(4,54) = 2.94$; $p = 0.02$] and on the treatment × trial interaction [$F(8,108) = 2.16$; $p = 0.03$]. *Post hoc* comparisons showed that imipramine ($^*p < 0.05$), DCM 10 mg/kg and 15 mg/kg ($^{**}p < 0.01$) decreased avoidance 2 latency compared to the control group, indicating an anxiolytic effect.

For the escape test, the RMANOVA showed a significant main effect on treatment [$F(4,54) = 13.13$; $p < 0.001$] and on the trials [$F(2,108) = 3.25$; $p = 0.04$], but not a significant effect on treatment × trial interaction [$F(8,108) = 0.68$; $p = 0.70$]. *Post hoc* comparisons showed that imipramine increased escape 1, 2 and 3 latencies ($^{***}p < 0.001$), DCM 10 and 15 mg/kg significantly increased escape 2 ($^*p = 0.05$ and $^{**}p < 0.01$, respectively) and DCM 15 mg/kg significantly increased escape 3 ($^{**}p < 0.01$) latencies compared to the control group, indicating a panicolytic effect.

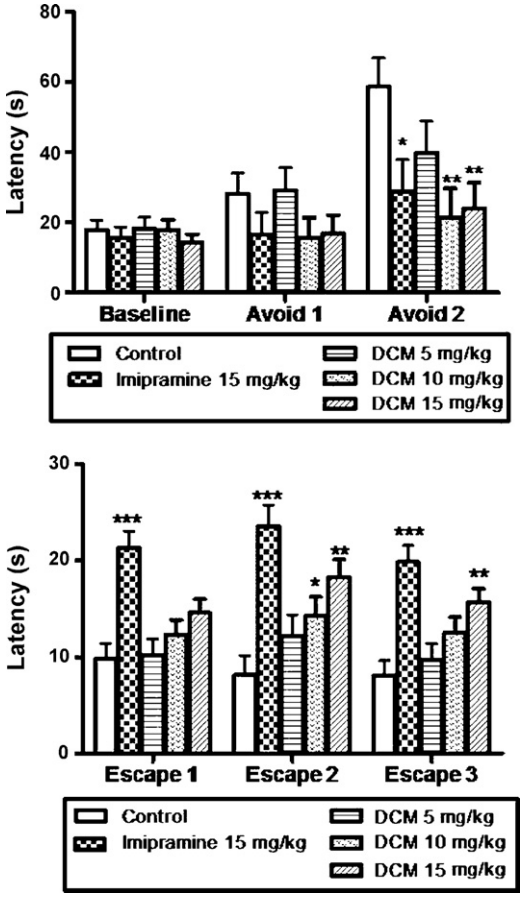

Fig. 2. Effects (mean ± SEM) of 21 days of administration of DCM or imipramine on inhibitory avoidance and escape latencies in the ETM test ($n = 10–15$). $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$ compared to the control group.

One-way ANOVA did not show significant differences in distance traveled in meters in the OFT ($p > 0.05$, $n = 10–15$) under the different treatments imipramine (15 mg/kg = 16.2 ± 1.2); DCM (5 mg/kg = 21.8 ± 1.2; 10 mg/kg = 16.5 ± 1.1; 15 mg/kg = 16.1 ± 1.0) compared to the control group (18.9 ± 1.1).

Discussion

This study evaluated the effects of DCM fraction on rats in the ETM test, an animal model developed to assess defensive behaviors related to specific subtypes of anxiety disorder, generalized anxiety disorder and panic disorder.

The data obtained showed that repeated treatment with DCM decreased inhibitory avoidance latency and increased escape latency without affecting the locomotion in the OFT, as occurs with the reference drug, imipramine. DCM was therefore found to have an anxiolytic and panicolytic effect on rats in the ETM test.

The phytochemical investigations on *Kielmeyera coriacea* led to the isolation and identification of ten xanthones, one biphenyl and two triterpenes (Cortez et al. 1998). Studies showed that 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone isolated from *Kielmeyera coriacea* and presented in DCM fraction is related with the antidepressant-like profile of this plant suggested by the anti-immobility effect in the FST (Martins et al. 2008; Sela et al. 2010).

Some studies show that xanthone derivatives (Ohishi et al. 2000) and xanthones present in the plant *Gentiana kochiana*, which show antidepressant therapeutic potential (Tomic et al. 2005), are able to inhibit monoamine oxidases (MAO), a mechanism in a class of antidepressants, establishing a relation between xanthones and antidepressant activity. Others studies also showed that xanthones are able to inhibit the type A and type B MAO (Suzuki et al. 1981) and according to pharmacological investigations xanthones must be involved in the antidepressant effect of a standardized *Hypericum perforatum* extract (Butterweck et al. 1997).

The DCM fraction of *Kielmeyera coriacea* was evaluated in a toxicological study and showed an LD_{50} of 1.503 mg/kg by oral route and 538.8 mg/kg by intraperitoneal route in mice, indicating the safety of acute and repeated oral administration of the DCM fraction of *Kielmeyera coriacea* stems, which can therefore be continuously used with safety (Obici et al. 2008).

In conclusion, the results of the present study demonstrate that DCM is active orally, and produces an anxiolytic and panicolytic effect on rats in the ETM test. The results suggest that DCM could be a useful drug in the treatment of mood disorders such as generalized anxiety and panic disorder.

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References

Astin, J.A., 1998. Why patients use alternative medicine: results of a national study. JAMA 279, 1548–1553.

Audi, E.A., Otobone, F., Martins, J.V.C., Cortez, D.A.G., 2002. Preliminary Evaluation of *Kielmeyera coriacea* leaves extract on central nervous system. Fitoterapia 73, 517–519.

Baldessarini, R.J., 2006. Drug therapy of depression and anxiety disorders. In: Brunton, L.L., Lazo, J.S., Parker, K.L. (Eds.), Goodman & Gilman's The Pharmacologic Basis of Therapeutic. McGraw-Hill, New York, pp. 445–449.

Cortez, D.A.G., Young, M.C.M., Marston, A., Wolfender, L., Hostettmann, K., 1998. Xanthones, triterpenes and a biphenyl from *Kielmeyera coriacea*. Phytochemistry 47, 1367–1374.

Butterweck, V., Lieflaender-Wulf, U., Winterhoff, H., Nahrstedt, A., 1997. Effects of the total extract and fractions of *Hypericum perforatum* in animal assays for antidepressant activity. Pharmacopsychiatry 30, 117–124.

Eisenberg, D.M., Davis, R.B., Ettner, S.L., Appel, S., Wilkey, S., VanRompay, M., Kessler, R.C., 1998. Trends in alternative medicine use in the United States 1990–1997. JAMA 280, 1569–1575.

Goulart, Y.C.F., Martins, J.V.C., Santos, A.R., Moreira, L.Y., Calixto, J.B., Cortez, D.A.G., Audi, E.A., 2007. Involvement of serotonin in the antidepressant-like effect of extract from *Kielmeyera coriacea* stems. Pharm. Biol. 45, 169–175.

Guerra, M., Nodari, R., 2001. Biodiversidade: aspectos biológicos, geográficos, legais e éticos. In: Simões, C., Sckenkel, E.P., Gosman, G., Mello, J.C.P., Mentz, L.A., Petrovick, P.R. (Eds.), Farmacognosia: da planta ao medicamento. UFRGS/UFSC, Porto Alegre/Florianópolis, pp. 13–26.

Henderson, M., Glozier, N., Elliott, K.H., 2005. Long term sickness absence. Brit. Med. J. 330, 802–803.

Henning, E.R., Turk, C.L., Mennin, D.S., Fresco, D.M., Heimberg, R.G., 2007. Impairment and quality of life in individuals with generalized anxiety disorder. Depress. Anxiety 24, 342–349.

Hoffman, E.J., Mathew, S.J., 2008. Anxiety disorders: a comprehensive review of pharmacotherapies. Mt. Sinai J. Med. 75, 247–262.

Kessler, R., Berglund, P., Demler, O., Jin, R., Merikangas, K., Walters, E., 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. Arch. Gen. Psychiatry 62, 593–602.

Martins, J.V.C., Otobone, F.J., Sela, V.R., Obici, S., Trombelli, M.A., Cortez, D.A.G., 2004. Activity of hydroethanolic extract from *Kielmeyera coriacea* stems on central nervous system in rats. Acta Sci. Health Sci. 26, 148–153.

Martins, J.V.C., Otobone, F.J., Sela, V.R., Obici, S., Trombelli, M.A., Garcia, D.A.G., Audi, E.A., 2006. Behavioral effects of *Kielmeyera coriacea* extract in rats. Indian J. Pharmacol. 38, 427–428.

Martins, J.V.C., Sela, V.R., Oliveira, S., Cortez, D.A.G., Audi, E.A., 2008. Serotonergic neurons of dorsal raphe nucleus on the effect of a xanthone from *Kielmeyera coriacea* stems in behavioral tests. Pharm. Biol. 46, 883–888.

Obici, S., Otobone, F.J., Sela, V.R., Ishida, K., Silva, J.C., Nakamura, C.V., Cortez, D.A.G., Audi, E.A., 2008. Preliminary toxicity study of dichloromethane extract of *Kielmeyera coriacea* stems in mice and rats. J. Ethnopharmacol. 115, 131–139.

Ohishi, N., Suzuki, T., Ogasawara, T., Yagi, K., 2000. Xanthone derivatives as inhibitors for monoamine oxidase. J. Mol. Catal. B Enzyme 10, 291–294.

Roncon, C.M., Almeida, C.B., Klein, T., Melo, J.C.P., Audi, E.A., 2010. Anxiolytic effects of a semipurified constituent of guaraná seeds on rats in the elevated T-maze test. Planta Med. 76, 1–6.

Schonfeld, W.H., Verboncoeur, C.J., Fifer, S.K., Lipschutz, R.C., Lubeck, D.P., Buesching, D.P., 1997. The functioning and well-being of patients with unrecognized anxiety disorders and major depressive disorder. J. Affect. Disord. 43, 105–119.

Sela, V.R., Hattanda, I., Albrecht, C.M., De Almeida, C.B., Obici, S., Cortez, D.A., Audi, E.A., 2010. Effect of xanthone from *Kielmeyera coriacea* stems on serotonergic neurons of the median raphe nucleus. Phytomedicine 17, 274–278.

Stein, M.B., Heimberg, R.G., 2004. Well-being and life satisfaction in generalized anxiety disorder: Comparison to major depressive disorder in a community sample. J. Affect. Disord. 79, 161–166.

Suzuki, O., Katsumata, Y., Oya, M., Chari, V.M., Vermes, B., Wagner, H., Hostettmann, K., 1981. Inhibition of type A and type B monoamine oxidases by naturally occurring xanthones. Planta Med. 42, 17–21.

Tomic, M., Tovilović, G., Butorović, B., Krstić, D., Janković, T., Aljančić, I., Menković, N., 2005. Neuropharmacological evaluation of diethylether extract and xanthones of *Gentiana kochiana*. Pharmacol. Biochem. Behav. 81, 535–542.