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Ex vivo and *in vitro* inhibitory potential of Kava extract on monoamine oxidase B activity in mice

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ABSTRACT

Background and aim: This study investigated the effect of Kava extract (*Piper methysticum*), a medicinal plant that has been worldly used by its anxiolytic effects, on monoamine oxidase (MAO) activity of mice brain after 21 days of treatment as well as anxiolytic and locomotor behavior. Furthermore, the *in vitro* inhibitory profile of Kava extract on MAO-B activity of mouse brain was evaluated.
Experimental procedure: Mice were treated with Kava extract (10, 40, 100 and 400 mg/kg) for 21 days by gavage. After behavioral analysis (plus maze test and open field), MAO activity in different mouse brain structures (cortex, hippocampus, region containing the substantia nigra and striatum) were performed. MAO-B inhibitory profile was characterized *in vitro*.
Results: The treatment with Kava extract (40 mg/kg) increased the percentage of entries of mice into the open arms. *Ex vivo* analysis showed an inhibition on MAO-B activity caused by Kava extract in cortex (10 mg/kg) and in the region containing the substantia nigra (10 and 100 mg/kg). *In vitro*, Kava extract also reversibly inhibited MAO-B activity with $IC_{50} = 14.62$ μg/mL and, increased K*m* values at the concentrations of 10 and 30 μg/mL and decreased V*max* value at 100 μg/mL.
Conclusion: Kava extract showed different effects on MAO-B isoform depending on the brain structure evaluated. Therefore, the use of Kava extract could be promissory in pathologies where MAO-B is the pharmacological target.

1. Introduction

The perennial shrub named Kava (*Piper methysticum* - G. Forst) is native from the South Pacific islands countries and, the herb extracted of its roots or rhizome has been used in modern phytotherapy as a recreational kava-drinking due to its relaxing properties and as a natural alternative to replace anxiolytic and sleeping drugs.[1,2] In addition to its anxiolytic/hypnotic effects, studies with Kava extract already showed analgesic and anticancer properties *in vitro* and *in vivo*, as well as improved of the psychotic symptoms.[3–5] The presence of different components in Kava extract have been reported. Among them fifteen kavalactones may be found in the crude extract, being six kavalactones the most abundant - kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin. In lower quantities, Kava chalcones (flavokavains A, B and C - a class of open chain flavonoids), amino acids, minerals and phenolic compounds are also present among the chemicals already isolated from the extract.[6–8] A variety of biological mechanisms of these secondary metabolites from Kava extract have been demonstrated, incluing positive modulation of gamma-aminobutyric acid type A ($GABA_A$) receptors, affinity by DA type-2 and opioid receptors, reduction in

Abbreviations: 4-HQ, 4-hydroxyquinoline; 5-HT, serotonin; AMPH, amphetamine; CEUA, Ethic Committee on Animal Use; CNS, central nervous system; CONCEA, National Council of Control of Animal Experimentation; DA, dopamine; GABA, gamma-aminobutyric acid; HPLC, High performance liquid chromatography; MAO, Monoamine oxidase; NE, norepinephrine.

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reuptake of neuronal norepinephrine (NE) and dopamine (DA), inhibition of voltage-gated Na^+ and Ca^{2+} ion channels, and inhibitory effects on monoamine oxidase -B (MAO-B) *in vitro*.[4,9,10]

MAO is a flavoenzyme found in the outer mitochondrial membrane of cells present in the central nervous system (CNS) and peripheral tissues of mammals.[11,12] It catalyzes the oxidative deamination of biogenic and dietary amines to their corresponding aldehydes with subsequent formation of hydrogen peroxide and ammonia.[13–15] MAO has two isoforms, MAO-A and MAO-B, which are differentiated by their specificities, substrates and inhibitors.[16,17] MAO-A isoform preferentially metabolizes hydroxylated amines as NE and serotonin (5-HT) and, it is selectively inhibited by clorgyline.[17,18] Whereas MAO-B metabolizes non-hydroxylated amines as benzylamine and beta-phenylethylamine and, it is selectively inhibited by rasagiline, pargyline and low concentrations of selegiline.[17,18] Amines as epinephrine, DA, tryptamine and tyramine are substrates for both MAO isoforms in the majority of the species.[19] In this sense, MAO inhibitors have a long-standing use as anti-parkinsonian and anti-depressant drugs that improve quality of life for patients.[20] Recently, Krum et al. demonstrated that Kava extract avoided the increase of stereotyped behavior in a model of psychosis-like symptoms induced by amphetamine (AMPH) in mice.[8] Furthermore, the acute administration of Kava extract inhibited MAO isoforms in cortex and hippocampus of mice.[8] Given the changes in MAO activity observed, we have now analyzed its effects on this enzyme after 21 days of treatment with Kava extract in mice.

Thus, the main goal of the present study was to evaluate the effect of 21 days of treatment with Kava extract on MAO-A and MAO-B isoforms in different mouse brain structures. Also, the behavioral changes with the doses used were evaluated as well as the *in vitro* inhibitory profile of Kava extract on MAO-B activity in mouse brain.

2. Material and methods

2.1. Plant material and chemicals

The crude extract of Kava rhizome (*Piper methysticum* – with approximately 30% of kavalactones) was obtained from Huakang Biotechnology Development (China - manufacturer's lot HK20160415). The composition of the extract was previously described by Krum et al.[8] All reagents were purchased from Sigma (Sigma-Aldrich, São Paulo, SP, Brazil) or other with high quality and purity.

2.2. Animals

Male Albino Swiss mice weighing between 25 and 35 g (2 months of age) from breeding colony of experimental house of Federal University of Santa Maria were used in this study. The animals were housed in polycarbonate cages (44 cm × 30 cm/4–5 per cage) with free access to water and food, in a temperature-controlled room (22 ± 2 °C) and under a 12-h light/dark cycle (lights on at 7:00 a.m.). All the animal experimentation was conducted in accordance with the guidelines of the National Council of Control of Animal Experimentation (CONCEA) and, the experimental procedures were approved by the Ethic Committee on Animal Use of Federal University of Santa Maria, Brazil under the protocol number CEUA 1637290415.

2.3. In vivo experimental design

Mice were randomly assigned into five groups (8 animals per group): (I) control, (II) Kava extract 10 mg/kg, (III) Kava extract 40 mg/kg, (IV) Kava extract 100 mg/kg and (V) Kava extract 400 mg/kg. Kava extract 40 mg/kg corresponds to the dose commonly used by humans (±200 mg/60 kg), which was calculated by allometric conversion.[21] Based on that, a pharmacological curve was carried out. Animals received Kava extract or its vehicle (corn oil) orally by gavage in a volume of 5 mL/kg once a day for 21 days.[22] Behavioral assessment was performed on day 22 of the experimental period (24 h after the last administration of Kava extract or vehicle) and all possible efforts were taken to avoid animal stress (environmental enrichment, low noise levels, appropriate supply of water, food, humidity, light, temperature, and others). After, the mice were euthanized by cervical dislocation in another experimental room and, rapidly, their brains were dissected in cortex, hippocampus, region containing the substantia nigra and striatum, frozen in powdered dry ice and stored at −80 °C for *ex vivo* analysis (MAO-A and MAO-B activity).

2.4. Behavioral assays

2.4.1. Elevated plus maze

Elevated plus maze test was conducted to evaluate the possible anxiolytic-like effect caused by Kava extract in mice.[23] The apparatus consisted of four arms, two open and two closed (30 cm L × 5 cm W and for closed arms, walls with 17 cm H) elevated 38.5 cm from the floor. The animals were placed in the center of the apparatus and the time spent and the number of entries into open or closed arms and, the number of head dips during 5 min were evaluated. The time spent on open arm and the number of entries into the open arms were calculated and demonstrated in percentage, as follows: time spent or number of entries into the open arm/total time or total number of the entries into closed and open arm X 100, respectively.[24]

2.4.2. Open field test

To verify possible changes in spontaneous locomotor and exploratory activity caused by Kava extract, mice were placed individually in the center of an open field arena (44 cm L × 44 cm W × 44 cm H) divided into 16 squares (4 rows of 4). The number of lines crossed (locomotor activity) and frequency of rearing (stand-up responses on two paws - exploratory activity) were measured during 5 min with no habituation period.[23,25,26]

2.4.3. Ex vivo MAO activity

To measure MAO activity *ex vivo*, mouse brain structures such as cortex, hippocampus, region containing the substantia nigra and striatum were homogenized in assay buffer (16.8 mM Na_2HPO_4, 10.6 mM KH_2PO_4, 3.6 mM KCl, pH 7.4) and, the homogenate was used to determine the protein quantity.[27] The reaction mixture containing brain structures homogenates (0.25 mg of protein) plus 250 nM pargyline (selective MAO-B inhibitor) or 250 nM clorgyline (selective MAO-A inhibitor) was pre-incubated at 37 °C for 20 min. After, kynuramine (60 μM) was added to start the reaction and incubated for additional 30 min at 37 °C. The reaction was stopped with 10% trichloroacetic acid (TCA). The samples were centrifuged at 500×*g* for 8 min. 1 mL of the supernatant was mixed with 1 mL of NaOH (1 N) and used to estimate the MAO-A and MAO-B activity. The product of reaction 4-hydroxyquinoline (4-HQ) was determined in fluorimeter at 315 nm for excitation and 380 nm for emission.[28] Results were expressed in nmol of 4-HQ per milligram of protein per minute.[8,29,30]

2.5. In vitro experimental protocol

For *in vitro* assays, the potential inhibitory effect of Kava extract on MAO activity (MAO-A and MAO-B) and the reversibility of MAO

activity inhibition were tested in total mouse brains. The extract was diluted in ethanol 1% (vehicle) and tested at different concentrations (10, 30, 100 μg/mL). A control without ethanol was used to verify if the ethanol *per se* could cause alterations in MAO activity.

2.5.1. In vitro MAO activity

Kava extract was tested for its *in vitro* inhibitory potential on MAO-A and MAO-B activity. Briefly, brains were homogenized in assay buffer (16.8 mM Na_2HPO_4, 10.6 mM KH_2PO_4, and 3.6 mM KCl, pH 7.4) and the homogenate was used for protein determination.[27] The activities of MAO-A and MAO-B isoforms were measured using 0.25 mg of protein in the presence of 250 nM clorgyline (selective MAO-A inhibitor) or 250 nM pargyline (selective MAO-B inhibitor) into the reaction mix along with different concentrations of Kava extract (10, 30, 100 μg/mL) or its vehicle. The mixture was pre-incubated at 37 °C for 20 min and then, 60 μM kynuramine (non-selective MAO substrate) was added to start the enzymatic reaction and incubated for additional 30 min at 37 °C. To stop the reaction, 10% TCA was added to the mixture. The samples were centrifuged at 500×*g* for 8 min. 1 mL of the supernatant was mixed with 1 mL of NaOH (1 N) and used to estimate the MAO-A and MAO-B activity. The product of reaction 4-HQ was determined in fluorimeter (315 nm for excitation and 380 nm for emission).[28] Results were expressed in nmol of 4-HQ per milligram of protein per minute.[30–32] The reversibility of MAO-B activity was carried out using the dialysis method.[33,34] Then, mixtures of outer buffer (16.8 mM Na_2HPO_4, 10.6 mM KH_2PO_4, 3.6 mM KCl, 1 mM dithiothreitol) and mice brain homogenates (0.25 mg of protein) in the absence or presence of Kava extract (100 μg/mL) were dialyzed at 25 °C for 24 h. For each 1 mL of mixture dialyzed, 40 mL of outer buffer was used. The outer buffer was replaced to a fresh buffer at 1, 2, 4 and 6 h after the start of dialysis. Nondialyzed mixtures were maintained under the same assay conditions. Dialyzed and non-dialyzed mixtures were simultaneously assayed for MAO-B activity as described above 24 h after the start of dialysis, to evaluate the reversibility of the inhibition produced by Kava extract. For kinetic experiments, different concentrations of kynuramine (1–100 μM) were used, and the MAO-B activity was determined in the absence or presence of different concentrations of Kava extract (10–100 μg/mL) to calculate K_m and V_{max} values.

2.6. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test when appropriate. All values are expressed as mean + standard error of the mean (SEM). IC_{50} value was calculated by nonlinear regression using sigmoidal dose–response with a variable slope equation, K*m* (μM) and V*max* (nmol/min/mg protein) values by nonlinear regression using the Michaelis–Menten equation. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Kava extract causes anxiolytic-like effect without altering locomotor and exploratory behavior in mice

In plus maze test, one-way ANOVA reveled a significant effect of Kava extract in the percentage of the number of entries into the open arms [$F(4, 35) = 3.82$; $p < 0.05$; Fig. 1B] *Post hoc* analysis showed Kava extract (40 mg/kg) increased the percentage of number of entries into the open arms compared to control group. Kava extract at 100 and 400 mg/kg decreased the percentage of the number of entries into the open arms when compared to Kava extract 40 mg/kg. However, no significant effect was detected in the percentage of time spent into the open arms and in the number of head dips (Fig. 1A and C, respectively) in plus maze test.

In open field test, Kava extract did not cause significant effects neither in the number of crossing nor in the number of rearing (Fig. 1D and E).

3.2. Kava extract decreases MAO-B activity in cortex and substantia nigra of mice

MAO-A and MAO-B activities were evaluated in cortex, hippocampus, region containing the substantia nigra and striatum of treated mice. For MAO-A activity, no significant difference among the groups was found in the brain structures analyzed (Fig. 2A, B, C, D). On the other hand, one-way ANOVA showed a significant effect of Kava on MAO-B activity in cortex [$F(4, 20) = 3.80$; $p < 0.05$; Fig. 3A] and in the region containing the substantia nigra [$F(4, 20) = 4.08$; $p < 0.05$; Fig. 3C]. Kava extract decreased the MAO-B activity in cortex at dose of 10 mg/kg, and in region containing the substantia nigra at doses of 10 and 100 mg/kg compared with their respective control group. No changes were observed in MAO-B activity in hippocampus and striatum (Fig. 3B and D).

3.3. Kava extract inhibits MAO-B activity with a reversible profile in vitro

One-way ANOVA showed a significant effect of Kava extract on MAO-A [$F(5, 12) = 5.16$; $p < 0.05$; Fig. 4A] and MAO-B activity [$F(5, 12) = 26.46$; $p < 0.05$; Fig. 4B]. *Post hoc* analysis demonstrated that Kava extract 100 μg/mL decreased MAO-A activity only when compared to the concentrations at 3 and 10 μg/mL of the extract. To MAO-B, *post hoc* analysis showed Kava extract decreased MAO-B activity at concentrations of 10, 30 and 100 μg/mL compared to control and vehicle groups with an $iC_{50} = 14.62$ μg/mL. Furthermore, Kava extract decreased MAO-B in a concentration-dependent manner since *post hoc* analysis showed the concentrations of 30 and 100 μg/mL were different from 3 μg/mL.

The reversibility of the MAO-B inhibition induced by Kava extract was measured by using the dialysis method. The inhibition caused by Kava extract at 100 μg/mL on MAO-B activity was completely reversible after dialysis [$F(1,12) = 13.67$; $p < 0.05$; Fig. 4C]. Lastly, in the kinetic experiments, Kava extract caused an increase in the K*m* values at 10 and 30 μg/mL (23.05 ± 3.61 and 40.01 ± 7.83 μM, respectively) and decrease V*max* values at 100 μg/mL (0.259 ± 0.022 nmol/min/mg of protein) compared to K*m* and V*max* values in the absence of Kava extract (12.63 ± 1.54 and 0.419 ± 0.014 nmol/min/mg of protein, respectively) (Fig. 4D and Table 1).

4. Discussion

Pharmacological actions in CNS have been demonstrated to Kava extract and, our previous study demonstrated that Kava extract decreases stereotyped behavior and promotes alterations in MAO activity in mice[8]; however, these effects on MAO activity have not been fully explored yet. Kava extract at 40 mg/kg caused an increase in the percentage of entries into the open arm in plus maze test without altering locomotor activity of the mice in the open field test. The treatment with Kava extract for 21 days reduced the activity of MAO-B in cortex at a dose of 10 mg/kg and in the region containing the substantia nigra at the doses of 10 and 100 mg/kg without altering the activity of MAO-A in any brain structure of mice. *In vitro*, Kava extract reversibly inhibited the MAO-B activity with an $IC_{50} = 14.62$ μg/mL, increasing the K*m* values at 10 and 30 μg/mL and decreasing the V*max* values at 100 μg/mL.

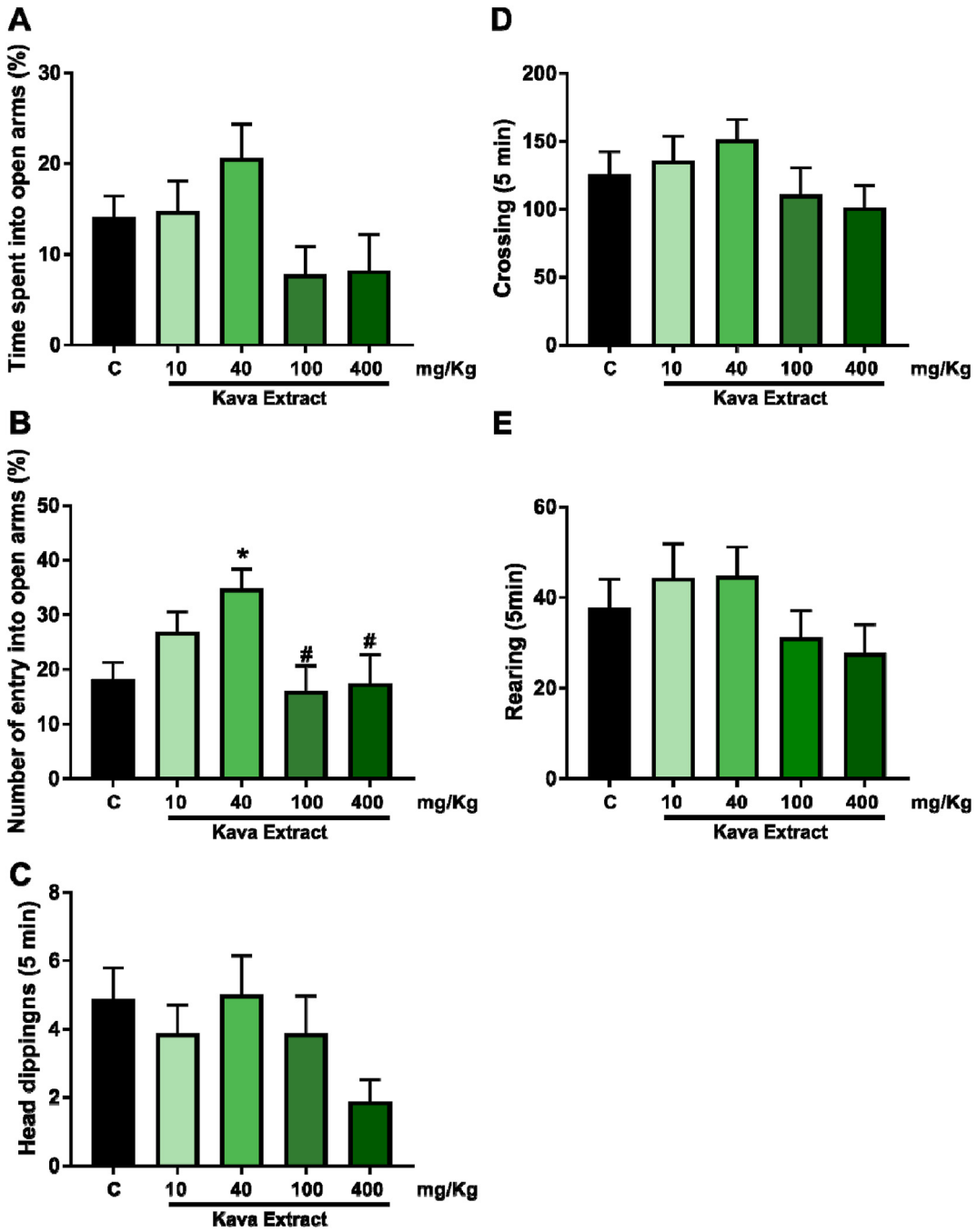

Fig. 1. Behavioral assays. (A) the percentage of the time spent on the open arms, (B) the percentage of the number of entries into the open arms and (C) the number of head dips in plus maze test, along with the number of (D) crossing and (E) rearing in the open field test were evaluated in mice, both for 5 min. The animals were treated with vehicle or Kava extract (10, 40, 100, 400 mg/kg) for 21 days. Data are expressed as mean + standard error of mean (n = 8). *$p < 0.05$, compared to control group. #$p < 0.05$, compared to kava extract (40 mg/kg) group.

The use of Kava extract in herbal medicine started in social ceremonies through Kava-drinking for centuries (approximately 3000 years ago).[35] The extract preparations (from parts of Kava plant) were made and used in local medicine for a range of illnesses such as sleeping difficulties, anxiety, convulsion, pain, fever, weight reduction, psychotic symptoms and others.[4,36,37] In the 1990s, Kava organic extract, now found in encapsulated and tincture forms, showed interest worldwide and it started to be used an over-the-counter preparations clinically proven treatment for anxiety, depression, insomnia and stress.[1,7,38] It is known that secondary metabolites of Kava extract such as kavalactones and chalcones have differents bioactivities. Kavain, for example, is implicated in a sudden relaxing effect while dihydrokavain and dihydromethysticin can cause nausea.[39] On the other hand, flavokavain A has been associated with anti-inflamatory and anticarcinogenic properties while flavokavain B with anti-inflamatory, anticarcinogenic, antinociceptive properties and hepatotoxicity.[39–41] In this context, *in vitro* studies demonstrated the six major kavalactones from Kava extract show MAO inhibition properties, which yangonin (with IC_{50} values of 1.29 μM/MAO-A and 0.085 μM/MAO-B) was the

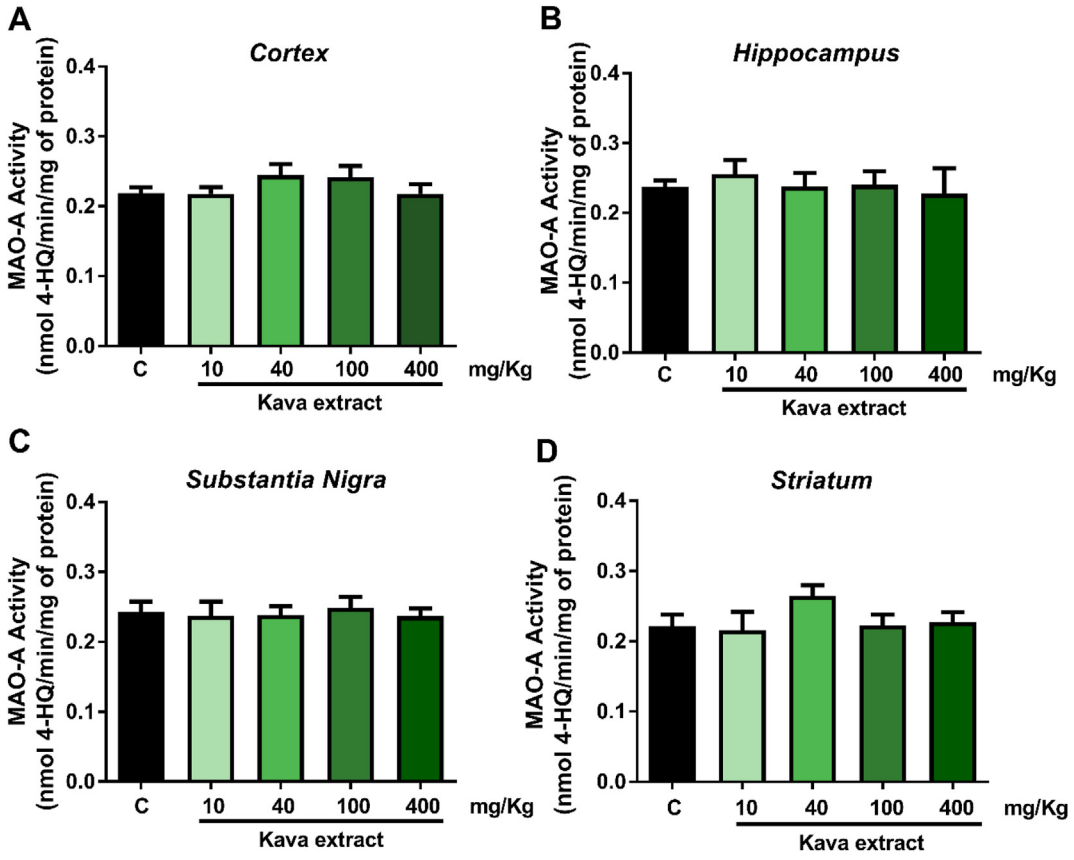

Fig. 2. MAO-A activity in (A) cortex, (B) hippocampus, (C) region containing the substantia nigra and (D) striatum in treated mice with vehicle or Kava extract (10, 40, 100, 400 mg/kg) for 21 days. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Data are expressed as mean + standard error of mean (n = 5).

most potent MAO inhibitor, followed by kavain (with IC_{50} values of 19 μM/MAO-A and 5.34 μM/MAO-B) and desmethoxyyangonin (with IC_{50} values of 4.44 μM/MAO-A and 2.51 μM/MAO-B).[42,43] Furthermore, other plant-derived metabolites may also show inhibitory activities on both isoforms of MAO, such as flavonoids, xanthones and alkaloids. The inhibitory activity on MAO enzyme of these secondary metabolites can be associated on their general structure activity relationship principles. The chalcones present in the Kava extract, have selectivity on MAO isoforms due substituents present in the B ring and double bond present in the 2 and 3 position of C ring, or electron donating hydrophilic hydroxyl group in the para position of B ring favoring the MAO-A inhibition, or absence of double bond between 2 and 3 positions of the C ring maintaining the non-planar nature of flavonoids and others [43].

Literature data also demonstrated that the dose used influences in the response produced by Kava on CNS of experimental animals.[44] In acute treatments, lower doses may produce mild stimulant effects while higher doses may cause sleepiness, ataxia and muscle relaxation.[44] Notwithstanding the Kava actions on GABAergic system are extensively studied and related to its main pharmacological use,[9,45] modulation on dopaminergic system[44,46] with consequent pharmacological effects were reported in humans[4,5,37] and experimental animals.[8] Recently, it was demonstrated that Kava reduced the stereotyped behavior induced by amphetamine in mice and had effects on MAO activity after a single administration of the extract[8] reinforcing the evidence of its action on other neurotransmitter systems beyond GABAergic system. However, of our knowledge, the effects of Kava extract on MAO activity *ex vivo* have not been fully investigated despite of *in vitro* evidence of its inhibitory effect on MAO-B.[10,42]

In the present study, mice were treated with Kava extract during 21 consecutive days to evaluate the effects of long-term treatment since there are few studies investigating the effects of Kava extract in chronic models. Firstly, we investigated the effects of Kava on behavioral effects in elevated plus maze test to evaluate if the doses used are promoting the anxiolytic effect which is reported in the literature.[1] Kava extract increased the percentage of entries in the open arms in elevated plus maze test at a dose of 40 mg/kg. Previously, Krum et al. demonstrated the single dose of 40 mg/kg also increased the number of entries of mice in the open field test[8] demonstrating this effect did not present tolerance with the repeated administrations at least up to 21 days of treatment. Interestingly, this dose corresponds to the equivalent dose used by humans calculated by allometric conversion (±200 mg/60 kg/day) for the relief of anxiety symptoms. Furthermore, the effects of Kava extract observed in plus maze test were not caused by an increase in locomotor activity since significant differences were not found in the open field test.

As it was previously demonstrated that Kava extract preferentially inhibited the activity of MAO-B *in vitro*[10,42] and, a single administration of Kava extract altered the MAO activity in brain regions of mice,[8] the main objective of the present study was to evaluate MAO-A and MAO-B activities in brain regions of mice treated with Kava extract for 21 days. Kava extract reduced the activity of MAO-B in the cortex at a dose of 10 mg/kg and in the region containing the substantia nigra at the doses of 10 and 100 mg/kg without altering the activity of MAO-A in any brain structure of mice. Interestingly, Kava extract seems to have different effects on MAO-B depending on the brain structure and time of treatment. Furthermore, the dose of Kava extract that

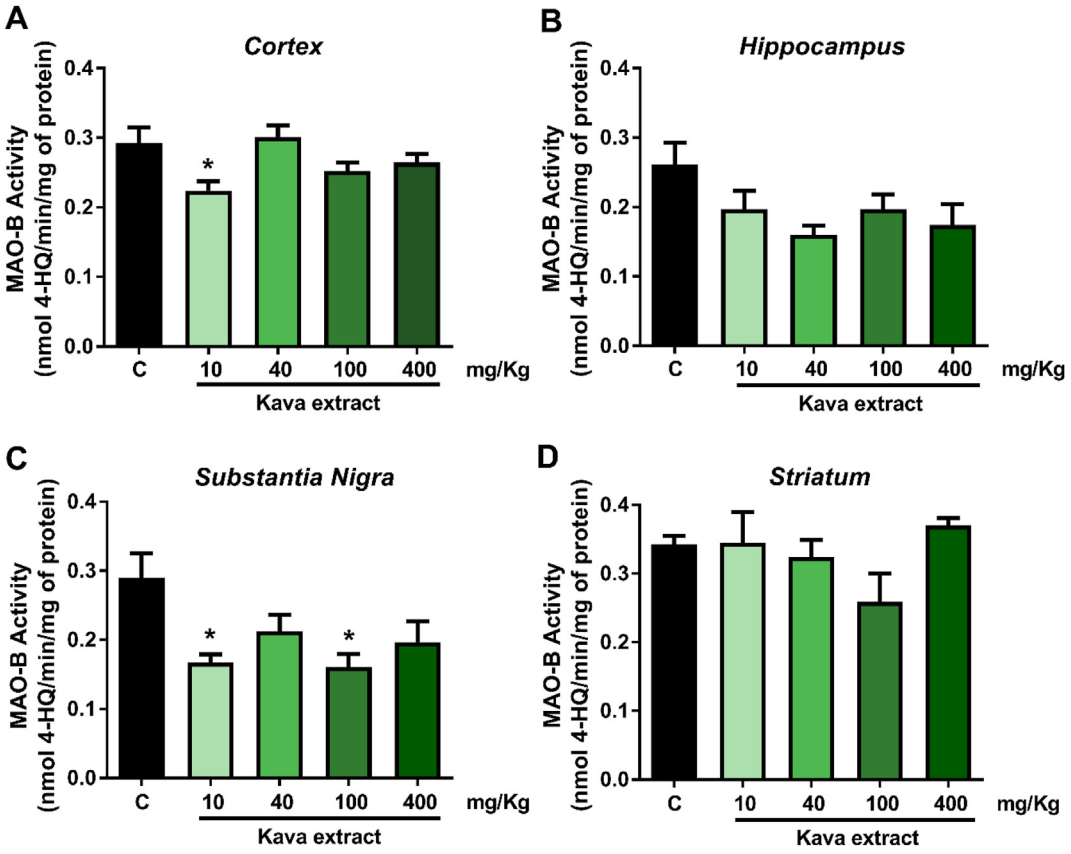

Fig. 3. MAO-B activity in (A) cortex, (B) hippocampus, (C) region containing the substantia nigra and (D) striatum in treated mice with vehicle or Kava extract (10, 40, 100, 400 mg/kg) for 21 days. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Data are expressed as mean + standard error of mean (n = 5). *$p < 0.05$, compared to control group.

showed anxiolytic affects was not the same dose that caused MAO-B inhibition. Krum et al.[8] demonstrated a decrease in MAO-A activity in cortex and MAO-B activity in hippocampus after a single dose of Kava extract in mice. Another important finding of the present study is the selectivity of Kava extract by MAO-B after long-term treatment once MAO-A inhibition is associated to higher probability of side effects.[19,47,48] Of our knowledge, this is the first study showing the inhibitory action of Kava extract *ex vivo* in a longer treatment in mice.

Considering it was found a reduction in the activity of MAO even after *ex vivo* analysis in the present and previous[8] studies from our group, an *in vitro* study was performed using the Kava extract to evaluate its effects on MAO activity from mice brain homogenates. Kava extract inhibited the activity of MAO-B with an iC_{50} of 14.62 μg/mL without altering the activity of MAO-A. The reversibility of the Kava extract binding to MAO-B was also confirmed since the percentage of activity was above of 80% after 24 h of dialysis[33] at the highest concentration tested (100 μg/mL). Moreover, Kava extract increased the K*m* values (10 and 30 μg/mL) and decreased the V*max* values (100 μg/mL) of MAO-B. Therefore, Kava extract was shown to be reversible and preferential MAO-B inhibitor. Our findings are in agreement with previous studies conducted by Uebelhack et al.[10] and Prinsloo et al.[42] Taken together, we suggest that the components of Kava extract can possess different bioavailability in the different regions of the brain when administered *in vivo*. Furthermore, even the binding of Kava extract with the enzyme is highly dynamic with reversible profile, a part of it continues bound during the *ex vivo* analysis maintaining the enzyme inhibited.

Monoamines are biological active compounds that act as neurotransmitters regulating several body functions such as behavioral, cognitive, motor and endocrine processes.[49] Biogenic amines are enzymatically metabolized by MAO which has participation in the regulation of their levels in mammals.[48,50,51] Currently, MAO inhibitors isocarboxazid, phenelzine, tranylcypromine, safinamide, rasagiline and selegiline are the medications approved by *Food and drug administration* (FDA) for treatment of depression, Parkinson's disease, and Alzheimer's disease.[52–55] Moreover, recent research with plant-derived compounds based on chalcones with pharmacophores from FDA approved drugs may become therapeutic possibility as MAO inhibitors.[54,56] In this sense, Kava chalcones (flavokavain A, B, C) could be contributing to the effects promoted by kavalactones, an MAO inhibitor in *in vitro* studies already known. However, there are still no studies about this possible effect.[42]

Previous studies have demonstrated the Kava extract can modulates monoamines as DA, NE, 5-HT in rodents[44] and zebrafish.[57] Furthermore, recently Krum et al.[8] demonstrated Kava extract decreased stereotyped behavior induced by psychostimulant amphetamine and MAO activity in mice suggesting a potential monoamine-modulating *in vivo*. Considering the reports of different pharmacological actions of Kava, its use should be cautious. In the same way that Kava extract could be used as a pharmacological agent (mainly as adjuvant – MAO-B inhibitor) for the treatment of pathologies as anxiety, Parkinson's disease, Huntington's disease, Alzheimer's disease, psychosis[4,8,18,19,37,50,58–60] it could be also used as abuse substance, since Kava extract has psychoactive effects by acting on the mesolimbic reward system.[57,61]

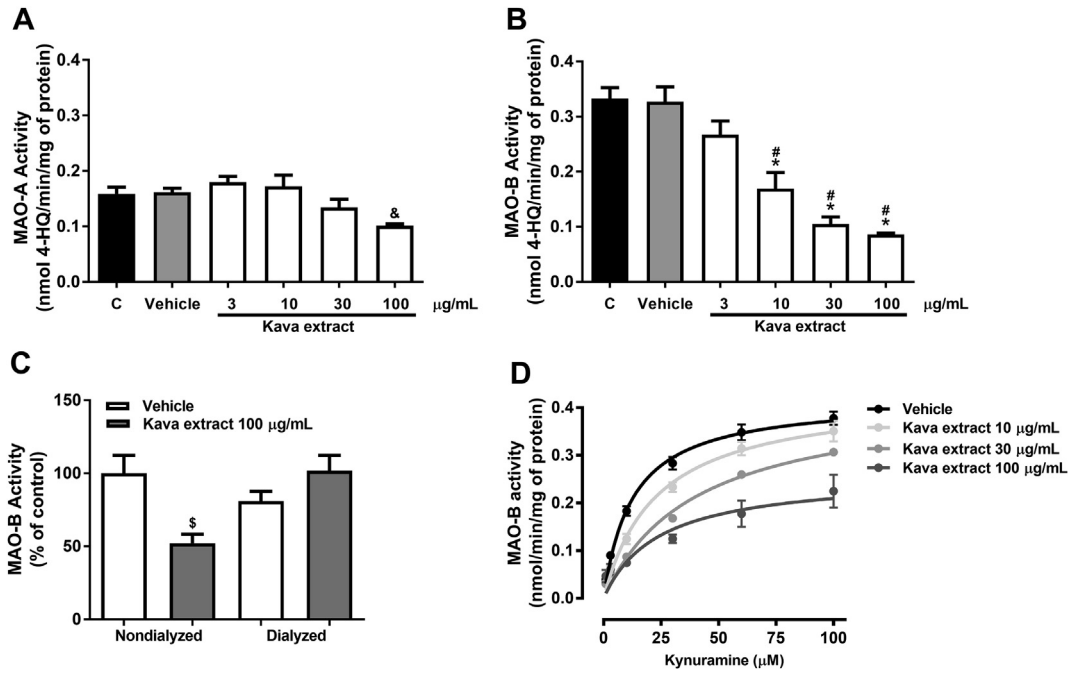

Fig. 4. Inhibitory potential *in vitro* of Kava extract (10, 30, 100 µg/mL) on (A) MAO-A and (B) MAO-B activities in mouse brain homogenates. Values are expressed as mean + standard error of mean (n = 3–4). MAO activity was analyzed by one-way followed by Tukey's post hoc test. $^{\&}p < 0.05$, compared to Kava extract 3 and 10 µg/mL $^{*}p < 0.05$, compared to control and ethanol group. $^{\#}p < 0.05$, compared to Kava extract 3 µg/mL (C) reversibility of MAO-B inhibition caused by Kava extract (100 µg/mL) after 24 h of dialysis in mouse brain homogenates. Values are expressed as mean + standard error of mean (n = 3–4). Reversibility was analyzed by two-way ANOVA followed by Tukey's post hoc test. $^{\$}p < 0.05$, compared to nondialyzed control. (D) Substrate concentrations curve for *in vitro* MAO-B activity in the absence or presence of Kava extract (10, 30, 100 µg/mL). K*m* (µM) and V*max* values were calculated by nonlinear regression using the Michaelis–Menten equation (n = 6).

Table 1
K*m* and V*max* values in the absence or presence of Kava extract for MAO-B activity from mouse brain homogenates.

	Kava extract (µg/mL)	K*m* (µM)	V*max* (nmol/min/mg of protein)
MAO-B	0	12.63 ± 1.54	0.419 ± 0.014
	10	23.05 ± 3.61*	0.429 ± 0.022
	30	40.01 ± 7.83*	0.426 ± 0.034
	100	23.84 ± 6.12	0.259 ± 0.022*

Data were analyzed by test-t. *p < 0.05 when compared with control group.

In conclusion, the present study demonstrated the potential action of Kava extract as an MAO-B inhibitor *ex vivo,* which showed different potencies depending on the analyzed brain structure. It was confirmed for *in vitro* assays, where Kava extract reveled also to be a reversible and preferential MAO-B inhibitor. Therefore, Kava seems to act on monoaminergic system in mice. Its effects could be promissory as adjuvant therapeutic approaches in pathologies involving alterations in MAO-B activity.

Author's contribution

Conception and design of the work: BNK and RF; conducted experiments: BNK, CMF, AB, LFS; data analysis and interpretation: BNK and RF; statistical analysis: BNK; writing–original draft preparation: BNK. The authors declare that all data were generated in-house and that no paper mill was used.

Declaration of competing interest

All authors declare there are no potential financial, personal, or otherwise conflicts of interest.

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