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Kava decreases the stereotyped behavior induced by amphetamine in mice

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ABSTRACT

Ethnopharmacological relevance: Kava extract (*Piper methysticum*) is a phytotherapic mainly used for the treatment of anxiety. Although the reported effects of Kava drinking improving psychotic symptoms of patients when it was introduced to relieve anxiety in aboriginal communities, its effects on models of psychosis-like symptoms are not investigated.

Aim of the study: To investigate the effects of Kava extract on behavioral changes induced by amphetamine (AMPH) and its possible relation with alterations in monoamine oxidase (MAO) activity.

Materials and methods: Mice received vehicle or Kava extract by gavage and, 2 h after vehicle or AMPH intraperitoneally. Twenty-five minutes after AMPH administration, behavioral (elevated plus maze, open field, stereotyped behavior, social interaction and Y maze) and biochemical tests (MAO-A and MAO-B activity in cortex, hippocampus and striatum) were sequentially evaluated.

Results: Kava extract exhibited anxiolytic effects in plus maze test, increased the locomotor activity of mice in open field test and decreased MAO-A (in cortex) and MAO-B (in hippocampus) activity of mice. Kava extract prevented the effects of AMPH on stereotyped behavior and, the association between Kava/AMPH increased the number of entries into arms in Y maze test as well as MAO-B activity in striatum. However, Kava extract did not prevent hyperlocomotion induced by AMPH in open field test. The social interaction was not modified by Kava extract and/or AMPH.

Conclusion: The results showed that Kava extract decreased the stereotyped behavior induced by AMPH at the same dose that promotes anxiolytic effects, which could be useful to minimize the psychotic symptoms in patients.

1. Introduction

Schizophrenia is a chronic psychiatric illness that affects approximately 1% of the population around the world (Millier et al., 2014; Mueser and McGurk, 2004; Saha et al., 2005). It is characterized by positive (hallucinations, delusions), negative (apathy, social withdrawal) and cognitive (cognitive impairment) symptoms (Brennan et al., 2013; Insel, 2010; Mueser and McGurk, 2004; Nagai et al., 2011). Although the etiology of schizophrenia remains not completely elucidated, it is considered a multifactorial neurodevelopmental disorder

Abbreviations: AMPH, amphetamine; ANOVA, analysis of variance; CEUA, Ethic Committee on Animal Use; TCA, trichloroacetic acid; CNS, central nervous system; CONCEA, National Council of Control of Animal Experimentation; DA, dopamine; GABA, γ-gamma-aminobutyric acid; HPLC, High-performance liquid chromatography; H_2O_2, hydrogen peroxide; 4-HQ, 4- hydroxyquinoline; 5-HT, serotonin; MAO, monoamine oxidase; NaOH, sodium hydroxide; NE, noradrenaline; SEM, standard error of mean.

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associated with genetic and environmental factors (Broome et al., 2005; Van Os et al., 2010). Dopamine (DA), glutamate, serotonin (5-HT) and γ-aminobutyric acid (GABA) are some of the neurotransmitters involved in schizophrenia (Brennan et al., 2013). The alterations in these neurotransmitter circuits have been associated with hyperactivation of the mesolimbic dopaminergic pathway and a decrease in the mesocortical dopaminergic pathway with consequent appearance of schizophrenia symptoms (Abi-Dargham et al., 2000; Kucinski et al., 2011; Janowsky and Risch, 1979; Lieberman et al., 1990).

Amphetamine (AMPH) is a psychostimulant drug that acts by increasing the synaptic levels of DA, 5-HT and noradrenaline (NE) in the central nervous system (CNS) (Tonge, 1974; Vogel et al., 1985). In rodents, AMPH is a pharmacological agent used as a tool to mimic psychosis-like symptoms, allowing a better understanding of its mechanisms as well as finding agents with therapeutic potential for schizophrenia (Ceretta et al., 2018; Featherstone et al., 2007; Jones et al., 2011). AMPH may act either by increasing the processes of release or decreasing the re-uptake and metabolism of monoamines (DA, 5-HT and NE) (Faraone, 2018; Heal et al., 2013). The monoamine oxidase (MAO) enzyme catalyzes the oxidative deamination of the monoamines in their corresponding aldehydes with formation of hydrogen peroxide (H_2O_2) and ammonia (Cohen et al., 2002; Vindis et al., 2001). Alterations in the levels and the consequent metabolism of monoamines are related to the appearance of various neurological diseases including schizophrenia (Meltzer and Stahl, 1976).

Piper methysticum is a perennial shrub from Piperaceae (pepper) family, also called Kava due to the presence of kavalactones, which are the main constituents of its extract (Rex et al., 2002; Sarris et al., 2012). The crude extract of Kava has been used as ceremonial and social drink in the Pacific islands and, in the phytotherapy, as an effective short-term treatment of anxiety (Sarris et al., 2011; Singh and Singh, 2002). Furthermore, it has other medicinal uses which include actions anti-stress and sedative (Singh and Singh, 2002). The main mechanism associated to the anxiolytic effects of Kava on CNS of mammals is through the $GABA_A$ modulation (Chua et al., 2016; Sarris et al., 2011). However, other targets to Kava extract were also demonstrated as binding DA type-2 receptor, blockage of voltage-gated sodium and calcium ion channels, reduction of the neuronal reuptake of DA and NE, as well as an MAO-B inhibitor (Cairney et al., 2002; Dinh et al., 2001a; Laporte et al., 2011; Ligresti et al., 2012; Uebelhack et al., 1998). Of particular importance to the present study, evidences in the literature showed the possible antipsychotic effects of Kava extract improving the psychotic symptoms of patients from aboriginal communities in north Australia from Oceania when the Kava drinking was introduced to relieve anxiety (Cawte, 1986). Corroborating, Kava reduced psychotic symptoms in patients (Cairney et al., 2002; Cawte, 1986) and caused motor alterations as dyskinesia which are clinical signs of central DA antagonism (Cairney et al., 2002; Schelosky et al., 1995). Experimental data demonstrated that Kava extract could alters the DA levels in the *nucleus accumbens* of rats (Sällström Baum et al., 1998b) and bind to DA type-2 receptor (Dinh et al., 2001a) suggesting the action of Kava components on dopaminergic system. Despite of case-related reporting the effects of Kava on psychotic symptoms in patients, its effects were not investigated in a model of psychosis-like symptoms induced by AMPH in rodents. Based on the above-mentioned evidence, the research for new therapeutic agents as well as possible pharmacological adjuvants to use in the treatment of schizophrenia is relevant. Thus, the present study aimed to investigate the effects of the crude extract of Kava on behavioral changes induced by AMPH in mice and whether these effects are associated with alterations in MAO activity.

2. Materials and methods

2.1. Drugs

The crude extract of Kava rhizome (*P. methysticum*) was obtained from Huakang Biotechnology Development (China-manufacturer's lot HK20160415) with approximately 30% of kavalactones (according to the supplier's report). High-performance liquid chromatography (HPLC) was used to characterize the compounds of the extract. All reagents were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA) or other with high quality and purity.

2.2. Quantification of phenolics and flavonoids compounds by HPLC-DAD

The quantification of phenolic and flavonoid compounds was carried out as previously described (Peroza et al., 2013).

2.3. Animals

Thirty-two male Albino Swiss mice (2 months of age, 25–35 g) from the Central Animal House-holding of Federal University of Santa Maria were used in this study. The animals were housed in polycarbonate cages (four or five per cage) with free access to water and food, in temperature-controlled room (22 ± 2 °C) and on 12-h light/dark cycle with light on at 7:00 a.m. All experiments were performed in accordance to 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.

2.4. Experimental design

Mice were randomly assigned to one of four groups (eight mice/group): (I) Control, (II) Kava extract, (III) AMPH, or (IV) Kava extract + AMPH. The animals were acclimated for 1 h to the behavioral room before starting the drugs administration. The animals of groups I and III received pretreatment with vehicle of Kava extract (corn oil) and the animals of groups II and IV received pretreatment with Kava extract (40 mg/kg) orally by gavage. Two hours after, the animals in groups I and II received vehicle of AMPH (NaCl 0.9%) and the animals in groups III and IV received 1.25 mg/kg of AMPH by an intraperitoneal injection (i.p.) (Behl et al., 2011; Ceretta et al., 2016; Figueira et al., 2015). To mimic what happens clinically, the dose administered of Kava extract to the animals was calculated by allometric conversion (Reagan-Shaw et al., 2007) and it corresponds similarly to the dose commonly used by humans (±200 mg/60 Kg). The behavioral evaluation began 25 min after the administration of AMPH or its vehicle as previously described Figueira at al (Figueira et al., 2015) with minor modifications. The animal received the drugs administration and passed by all behavioral tests during approximately 40 min in the following sequence: Elevated plus maze, Open field test, Stereotypy, Social interaction test and Y maze test. Then, the total time that the animal was maintained in the experiment considering the acclimation, drug administration, behavioral analysis until euthanasia was approximately 4.5 h. During the experimental assays, all possible efforts were made to avoid animal stress as appropriate supply of water, food, temperature, humidity and light, environmental enrichment, low noise levels and others. Immediately after behavioral analysis, the animals were conducted to another experimental room and euthanized by cervical dislocation; their brains were rapidly dissected in cortex, hippocampus and striatum. The brain structures were frozen in powdered dry ice and, then stored at −80 °C to perform the MAO activity. The experimental design is depicted in Fig. 2.

2.5. Behavioral analysis

For the behavioral observations, the experimenters were blind regarding to the treatment conditions. The inter-rater reliability between 2 observers is greater than 90%. The calculated α value was significant for $p < 0.05$.

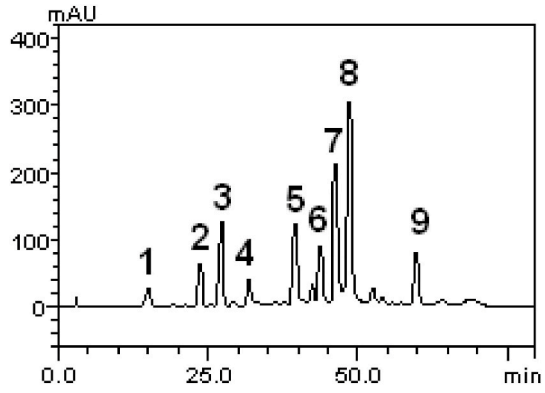

Fig. 1. High performance liquid chromatography profile of Kava extract. Panel A: Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rosmarinic acid (peak 4), rutin (peak 5), isoquercitrin (peak 6), quercitrin (peak 7), quercetin (peak 8) and kaempferol (peak 9).

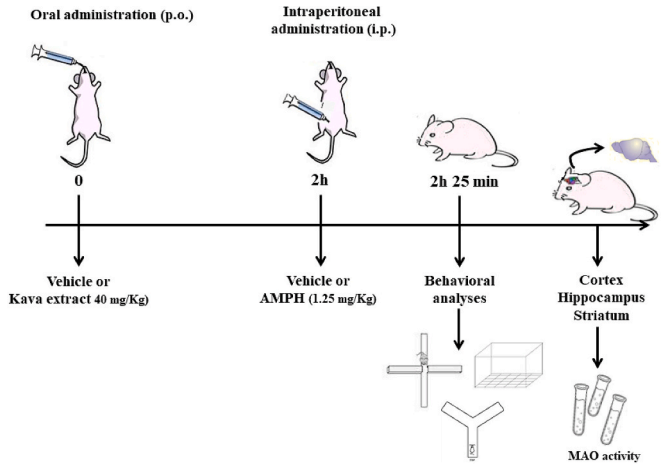

Fig. 2. Experimental design of the acute treatment with Kava extract and/or AMPH. Firstly, mice received 40 mg/kg of Kava extract or vehicle by gavage and, after 2 h 1.25 mg/kg of AMPH or vehicle intraperitoneally. Twenty-five minutes after last administration of AMPH or vehicle, behavioral analyses were performed. Brain dissections were carried out after euthanasia of animals to perform MAO activity. AMPH: amphetamine.

2.5.1. Elevated plus maze

To evaluate the anxiety-like state caused by treatments, mice were exposed to an elevated plus maze (Anchan et al., 2014; Treit et al., 1993). The apparatus consisted of four arms, two open and two closed arms (30 cm L × 5 cm W). For closed arms, walls (17 cm H) were positioned 38.5 cm from the floor. The animals were placed in the center of the apparatus to quantify the time spent into open or closed arms and the number of head dips during a 5 min session. The time spent on open arm and the entries into the open arms were calculated and expressed 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 (Fachinetto et al., 2007).

2.5.2. Open field test

Open field test was performed to verify possible changes in spontaneous locomotor and exploratory activity in treated mice (Anchan et al., 2014; Archer, 1973). The animals were placed individually in the center of an open field arena (44 cm L × 44 cm W × 44 cm H) divided into 16 equal areas. The number of lines crossed (locomotor activity), frequency of rearing (exploratory activity) and number of times the animal crosses the central square were measured during 5 min without habituation period.

2.5.3. Stereotypy

To evaluated stereotyped behavior in treated mice, the animals were placed in glass cages (20 cm L x 20 cm W x 19 cm H) and during 5 min the following parameters were taken to account stereotypy score: sniffing, grooming, nail biting, circling (Figueira et al., 2015; Machado et al., 2006). Scores from 0 to 4 were attributed for these parameters, as follow: 0) absence or 1) presence of one type of abnormal movements; 2) presence of two types of abnormal movements; 3) presence of three types of abnormal movements; 4) presence of four types of abnormal movements.

2.5.4. Social interaction test

Social behavior of mice treated with Kava extract and/or AMPH was performed in an open field arena (44 cm L × 44 cm W × 44 cm H) with a floor divided into 16 equal areas during 15 min. Pairs of unfamiliar mice receiving the same treatment were placed on opposite sides into the arena and the time of social interaction active (sniffing and following) or passive (when animals lie next to each other within a distance of 5 cm from skin to skin) and number of contacts of the animals treated were quantified as previously described by Calzavara et al. (2011) with some modifications.

2.5.5. Y maze test

Spatial working memory of mice treated with Kava extract and/or AMPH was evaluated by Y maze test. The apparatus consisted of three equal arms (A, B and C arms) (30 cm L x 6 cm W x 2 cm H) positioned 31 cm from the floor. The animals were individually placed at the end of the A arm and then the sequence in which the animal goes through the arms was quantified during 8 min. The series of arm entries (considered complete when the hind paws of the mice had been completely placed in the arm) was recorded visually. Correct alternation was defined as successive entries into the three different arms, on overlapping triplet sets for example ABC, BCA, CAB. The percentage of alternation was calculated as total of alternations/(total arm entries − 2) x 100 (Ceretta et al., 2016; Dall'Igna et al., 2007; Monte et al., 2013).

2.6. Tissue preparation and MAO activity

To carry out MAO activity *ex vivo* the brain structures 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 as previously described Lowry (1951). The reaction mixture containing brain homogenates (0.25 mg of protein), 250 nM pargyline (selective MAO-B inhibitor) or 250 nM clorgyline (selective MAO-A inhibitor) was pre-incubated at 37 °C during 20 min. The reaction was started by the addition of 60 μM kynuramine in the reaction mixture and incubated for more 30 min at 37 °C. The reaction was stopped with 10% trichloroacetic acid (TCA). The samples were centrifuged at 500*g* for 8 min and 1 mL of the supernatant was mixed with 1 mL of NaOH (1 N) (Busanello et al., 2017; de Freitas et al., 2018; Soto-Otero et al., 2001). The product of the reaction was measured in fluorimeter at 315 nm for excitation and 380 nm for emission by measuring the kynuramine oxidation to 4-hydroxyquinoline (4-HQ) (Morinan and Garratt, 1985). Results were expressed in nmol of 4-HQ per milligram of protein per minute.

2.7. Statistical analysis

Data were expressed as mean + standard error of the mean (SEM) and analyzed by two-way analysis of variance (ANOVA) (with AMPH and Kava as factors) followed by Tukey's *post hoc* test when appropriate. Data were considered statistically significant when $p < 0.05$.

3. Results

3.1. HPLC analysis

HPLC fingerprinting of the Kava extract showed an elution diagram when the peaks were grouped into three regions based on the UV absorption profile. These regions showed typical patterns of UV absorption, supporting the presence of gallic acid (14.35min; peak 1), chlorogenic acid (23.98 min; peak 2), caffeic acid (27.15 min; peak 3), rosmarinic acid (32.54 min; peak 4), rutin (39.08min; peak 5), isoquercitrin (44.26; peak 6), quercitrin (46.35min; peak 7), quercetin (49.13min; peak 8) and kaempferol (60.02min; peak 9), (Fig. 1 and Table 1).

3.2. Effects of Kava extract and/or AMPH on elevated plus maze test in mice

In plus maze test, Kava treatment induced a change in behavior since two-way ANOVA revealed a significant main effect in the percentage of time spent into the open arms [(1,28) = 33.82 and $p < 0.05$; Fig. 3A], number of entries into the open arms [(1,28) = 19.40 and $p < 0.05$; Fig. 3B] and in the number of head dips [(1,28) = 21.97 and $p < 0.05$; Fig. 3C]. The treatment with AMPH altered the behavior regardless Kava pre-treatment in the percentage of time spent into the open arms [(1,28) = 9.11 and $p < 0.05$; Fig. 3A] and in the number of head dipping [(1,28) = 5.71 and $p < 0.05$; Fig. 3B] without significant effects in the number of entries in the open arms [(1,28) = 3.50 and $p = 0.071$; Fig. 3B].

3.3. Effects of Kava extract and/or AMPH on open field test in mice

In open field test, two-way ANOVA revealed a significant main effect of Kava in the number of crossings [(1,28) = 11.43 and $p < 0.05$; Fig. 4A], rearings [F (1, 28) = 15.80; $p < 0.05$; Fig. 4B] and in the number of entries into the center of arena [F (1, 28) = 20.23; $p < 0.05$; Fig. 4C]. Two-way ANOVA also revealed a significant main effect of AMPH in the number of crossings in the open field arena [(1,28) = 6.22 and $p < 0.05$; Fig. 4A].

3.4. Effects of Kava extract and/or AMPH on stereotypy in mice

Two-way ANOVA revealed a significant main effect of Kava [F (1, 28) = 14.40; $p < 0.05$; Fig. 5], AMPH [F (1, 28) = 10.00; $p < 0.05$; Fig. 5] and the interaction [F (1, 28) = 10.00; $p < 0.05$; Fig. 5]. *Post hoc* analysis demonstrated that AMPH increased the stereotypy in mice compared with control group and the pre-treatment with Kava avoided the AMPH effects. Kava *per se* did not altered the stereotyped behavior in mice (Fig. 5).

Table 1
Phenolics and flavonoids composition of Kava extract.

Compounds	Kava extract		LOD	LOQ
	mg/g	Percent	μg/mL	μg/mL
Gallic acid	23.75 ± 0.03 a	2.37	0.017	0.056
Chlorogenic acid	50.93 ± 0.01 b	5.09	0.038	0.125
Caffeic acid	85.68 ± 0.02 c	8.56	0.031	0.102
Rosmarinic acid	21.16 ± 0.07 a	2.11	0.014	0.047
Rutin	87.49 ± 0.06 c	8.74	0.023	0.075
Isoquercitrin[a]	63.17 ± 0.04 d	6.31	-	-
Quercitrin[a]	112.45 ± 0.05 e	11.24	–	–
Quercetin	152.68 ± 0.01 f	15.26	0.007	0.023
Kaempferol	61.73 ± 0.03 d	6.17	0.041	0.135

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$.

[a] Quantified as quercetin.

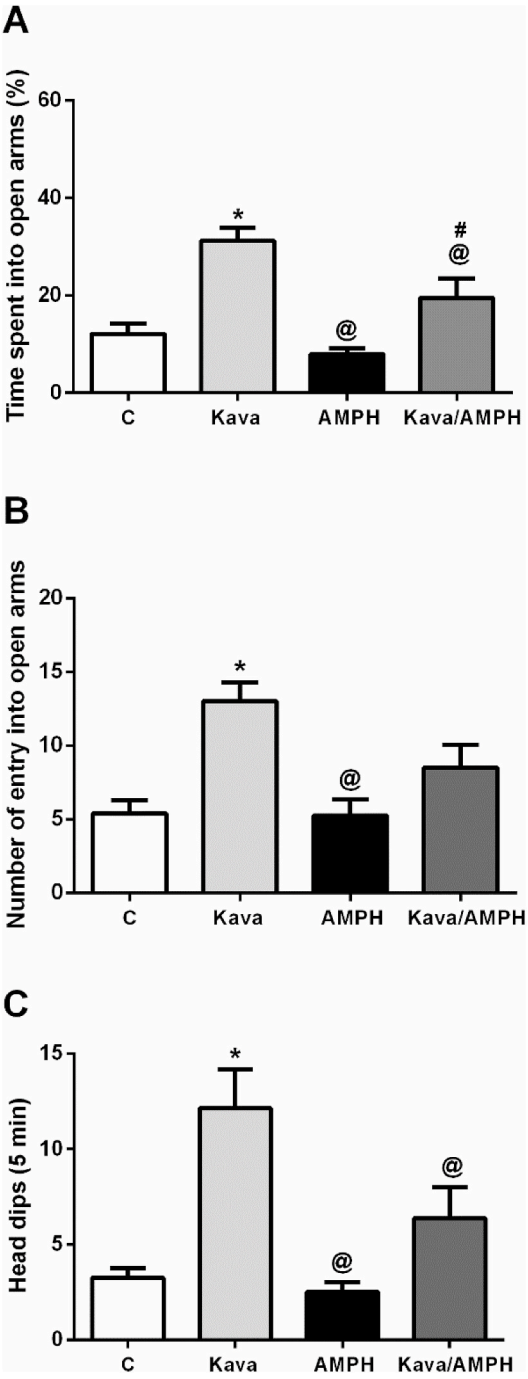

Fig. 3. Plus maze test. (A) Percentage of the time spent on the open arms, (B) number of entries into the open arms and (C) number of head dips in mice treated with Kava extract and/or AMPH during 5 min. Data were analyzed by two-way ANOVA followed by Tukey's *post hoc* test. Data are expressed as mean + standard error of mean (n = 8). $^{*}p < 0.05$, when compared with control group. $^{@}p < 0.05$, when compared with Kava group. $^{\#}p < 0.05$, when compared with AMPH group. C: control, AMPH: amphetamine.

3.5. Effects of Kava extract and/or AMPH on social interaction test in mice

Regarding social interaction test, statistical analysis did not found alterations neither in number of contacts nor in time of social interaction in mice treated when compared with control group (Fig. 6).

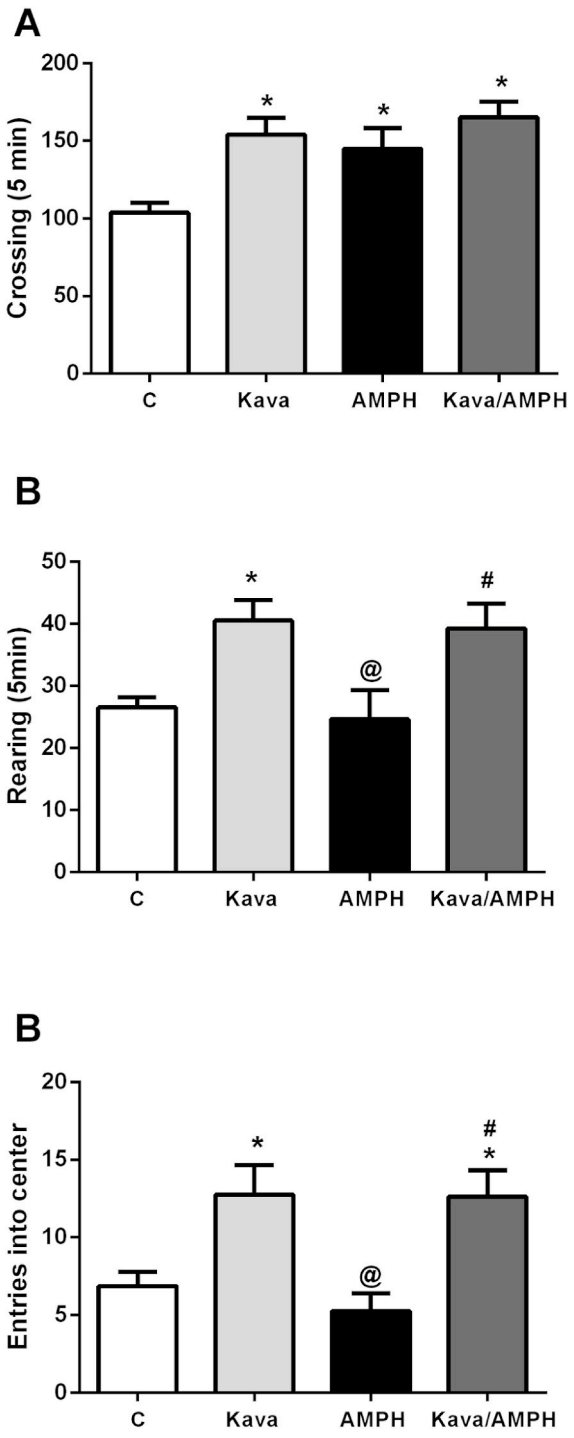

Fig. 4. Open field test. Number of (A) crossing and (B) rearing in mice treated with Kava extract and/or AMPH during 5 min. Data were analyzed by two-way ANOVA followed by Tukey's *post hoc* test. Data are expressed as mean + standard error of mean (n = 8). $^{*}p < 0.05$, when compared with control group. $^{@}p < 0.05$, when compared with Kava group. $^{\#}p < 0.05$, when compared with AMPH group. C: control, AMPH: amphetamine.

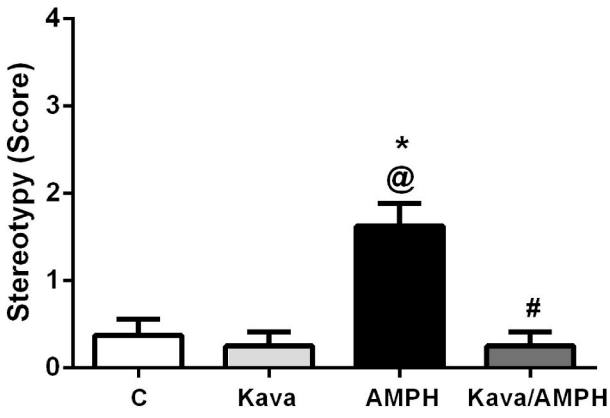

Fig. 5. Stereotypy. Score from 0 to 4 were attributed for stereotyped behaviors (sniffing, grooming, nail biting and circling) in mice treated with Kava extract and/or AMPH during 5 min. Scores: 0) absence or 1) presence and one type of abnormal movements; 2) presence and two types of abnormal movements; 3) presence and three types of abnormal movements; 4) presence and four types of abnormal movements. Data were analyzed by two-way ANOVA followed by Tukey's *post hoc* test. Data are expressed as mean + standard error of mean (n = 8). $^{*}p < 0.05$, when compared with control group. $^{@}p < 0.05$, when compared with Kava group. $^{\#}p < 0.05$, when compared with AMPH group. C: control, AMPH: amphetamine.

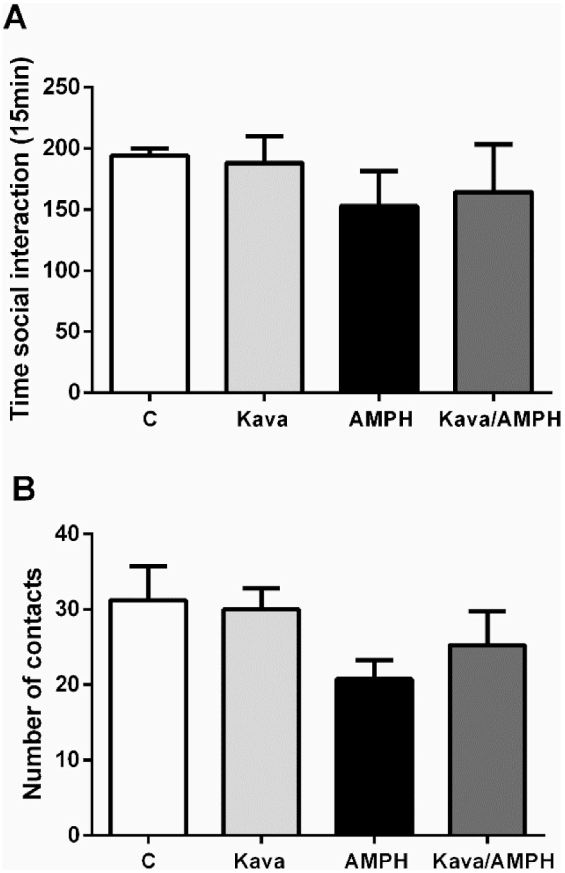

Fig. 6. Social interaction test. In open field arena the (A) time social interaction and (B) number of contacts in mice (animals tested in pairs) treated with Kava extract and/or AMPH during 15 min. Data were analyzed by two-way ANOVA followed by Tukey's *post hoc* test. Data are expressed as mean + standard error of mean (n = 4). AMPH: amphetamine.

3.6. Effects of Kava extract and/or AMPH on Y maze test in mice

Spatial working memory of mice treated with Kava extract and/or AMPH was evaluated in the Y maze test (Fig. 7). There were no significant effects of Kava extract and AMPH treatments in the percentage of correct alternations (Fig. 7A). A significant main effect of Kava was found in the number of entries into the arms [$F(1, 28) = 5.99$; $p < 0.05$;

Fig. 7B]. AMPH did not present significant effects on the number of entries into the arms [F (1,28) = 3.24 p = 0.08; Fig. 7B].

3.7. Effects of AMPH in mice pretreated with Kava extract on MAO activity in the cortex, hippocampus and striatum

MAO-A and MAO-B activity were evaluated in cortex, hippocampus and striatum of mice. A significant main effect of Kava was detected on MAO-A activity in cerebral cortex [F (1, 28) = 6.31; $p < 0.05$; Fig. 8A] as well as in MAO-B activity in hippocampus [F (1, 28) = 5.18; $p < 0.05$; Fig. 8E]. In striatum, a main effect of AMPH [F (1, 28) = 11.51; $p < 0.05$; Fig. 8E] was observed to MAO-B activity. No changes were observed in MAO-A activity in hippocampus and striatum even as for MAO-B activity in cortex (Fig. 8B, C, D).

4. Discussion

The present study aimed to investigate whether the crude extract of Kava could protect against behavioral alterations induced by AMPH in mice and, whether changes in MAO activity could be involved in its effects. The present results showed that AMPH produced an increase in behavioral responses as locomotor activity and stereotyped behavior without altering social interaction and spatial working memory. The pre-treatment with Kava extract avoided the increase of stereotyped behavior but did not prevent against hyperlocomotion induced by AMPH in mice. Moreover, the pre-treatment with Kava extract was associated with an increase in the number of entries into arms in Y maze test in those animals that received AMPH. Kava extract increased the frequency and the percentage of time spent on the open arm of the elevated plus maze as well as on the number of head dipping. It also increased locomotor and exploratory activity with an increase in the number of entries into the center of the open field. Kava extract decreased the activity of MAO-A in cortex and MAO-B in hippocampus, while AMPH increased the MAO-B activity in the striatum of animals pre-treated with Kava extract.

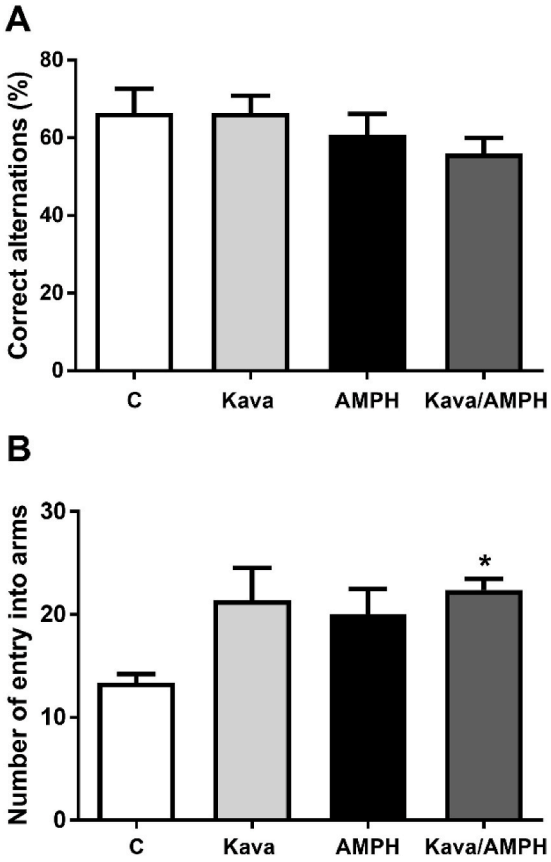

Fig. 7. Y maze test. Analyses of spatial working memory by (A) percentage of correct alternations and (B) number of entry into arms in mice treated with Kava extract and/or AMPH during 8 min. Data were analyzed by two-way ANOVA followed by Tukey's *post hoc* test. Data are expressed as mean + standard error of mean (n = 8). *$p < 0.05$, when compared with control group. C: control, AMPH: amphetamine.

Schizophrenic patients frequently present additional conditions as anxiety and major depression associated with psychotic symptoms (Sim et al., 2006). Approximately 65% of patients with schizophrenia exhibit anxiety symptoms (Sim et al., 2006). In this context, Kava extract could also play an important role in alleviating these symptoms due its action on GABA receptors (Laporte et al., 2011; Rex et al., 2002; Sarris et al., 2012; Singh and Singh, 2002) since GABAergic interneurons of ventral tegmental area are involved in the control of dopaminergic neurons from mesolimbic pathway (Yang and Tsai, 2017). The possible anti-psychotic effect of Kava was suggested in aboriginal communities in north Australia from Oceania when the Kava drinking was introduced to relieve anxiety and improved the psychotic symptoms of patients (Cawte, 1986). Furthermore, case-related reported the reduction of psychotic symptoms in patients (Cairney et al., 2002; Cawte, 1986) and appearance of motor alterations as dyskinesia in patients taking Kava extract suggesting its pharmacological effects as dopaminergic antagonist (Cairney et al., 2002; Schelosky et al., 1995). Despite of the mentioned clinical evidences and a study showing the Kava extract can bind in the type 2 dopaminergic receptors (Dinh et al., 2001a), its effects were not investigated in a model of psychosis-like symptoms induced by AMPH in rodents.

The present study was the first to investigate the effects of Kava extract on behavioral alterations induced by AMPH in mice as well as its possible effects on MAO activity. In this context, firstly we performed plus maze test to confirm the effectiveness of Kava extract, since this test evaluates anxiolytic-like behaviors in the animals (Anchan et al., 2014; Treit et al., 1993). As results, Kava extract induced a change in behavior increasing the percentage of time spent into the open arms, number of entries into the open arms and in the number of head dips, confirming that Kava extract at the dose used was reaching the CNS leading to an anxiolytic-like behavior. AMPH altered the behavior regardless Kava pre-treatment in the percentage and frequency of time spent into the open arms and in the number of head dipping. After, we carried out specific behaviors that could be modified in animal models of psychosis-like symptoms (Pogorelov et al., 2017).

In open field test, Kava extract increased the number of crossings, rearings and the number of entries into the center of open field. Confirming the anxiolytic effect of Kava (Kraeuter et al., 2019), the animals that received Kava extract presented a high number of entries into the center of open field. AMPH also increased the number of crossings in the open field arena and this effect was not modified by pre-treatment with Kava extract. The increase of locomotor activity caused by Kava extract *per se* suggests that it could be interacting with the same pathway of AMPH, which is demonstrated by the associated group Kava/AMPH that remained unchanged even with the combination of the two compounds. When the stereotyped behavior was evaluated, the administration of AMPH increased the stereotyped behavior of the mice and the pre-treatment with Kava extract prevented the appearance of these symptoms. These results suggest a decrease in dopaminergic activity. Both, hyperlocomotion and stereotyped behaviors (represented by unusual behaviors such as sniffing, grooming, nail biting, circling and immobility) in the animals, are associated to an hyperdopaminergic state in the mesolimbic pathway (mainly in the *nucleus accumbens*) (Ceretta et al., 2016; Ellenbroek and Cools, 2006; Featherstone et al., 2007; Figueira et al., 2015; Robinson and Becker, 1986; Saito et al., 2014; Wolgin, 2012). Taken together, it is hypothesized that either direct and/or indirect dopaminergic mechanisms are contributing to these behavioral effects. This hypothesis is based in the studies of

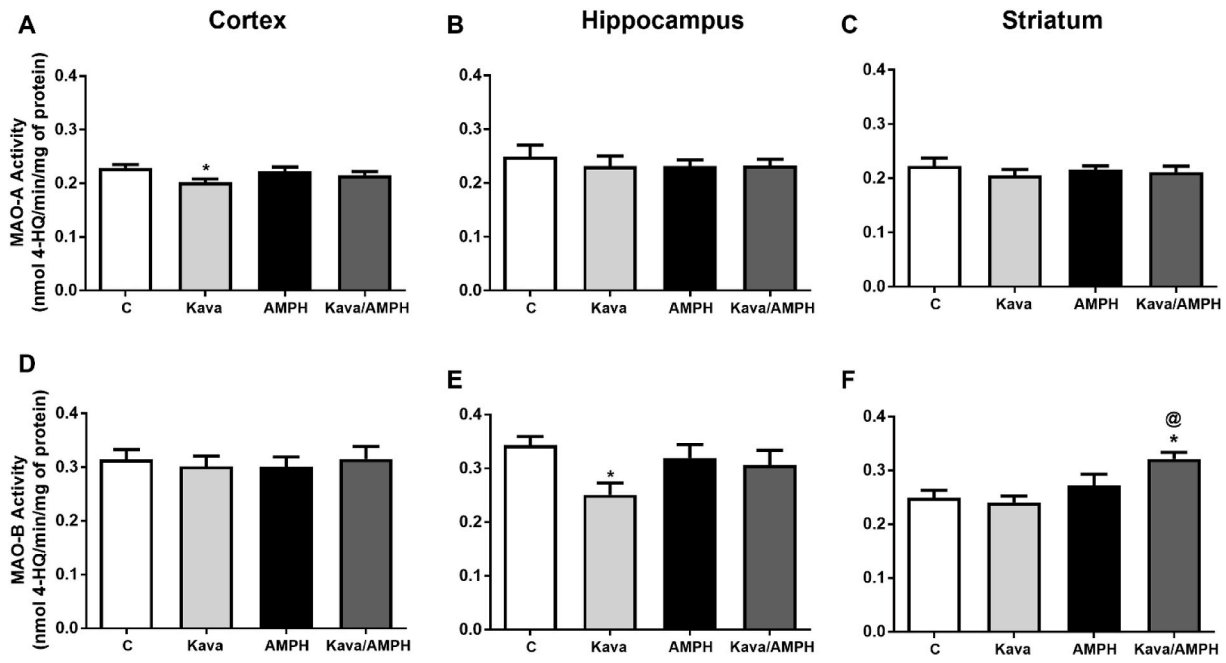

Fig. 8. MAO-A (A–C) and MAO-B activity (D–F) in cortex (A, D), hippocampus (B, E) and striatum (C, F) of mice treated with Kava extract and/or AMPH. Kynuramine was used as substrate and clorgyline (MAO-A) and pargyline (MAO-B) as a selective inhibitor. Data were analyzed by two-way ANOVA followed by Tukey's *post hoc* test. Data are expressed as mean + standard error of mean (n = 8). $^{*}p < 0.05$, when compared with control group. $^{@}p < 0.05$, when compared with Kava group. C: control, AMPH: amphetamine.

Sällström Baum et al., which demonstrated that Kava extract can cause an increase or decrease in DA levels in *nucleus* accumbens of rats (Sällström Baum et al., 1998) as well as the inhibitory potential on MAO-B (Prinsloo et al., 2019) and the binding with the type 2 dopaminergic receptors (Dinh et al., 2001b) *in vitro*. All these effects vary depending on the dose and the parts of plant used to prepare Kava extract (Dinh et al., 2001a; Sällström Baum et al., 1998). Regarding the differential effects of Kava in hyperlocomotion and stereotyped behavior, similar effects were observed when tested diphenyl diselenide (Figueira et al., 2015) and gabapentin (Ceretta et al., 2016) in AMPH model. Diphenyl diselenide avoided the effects of AMPH on number of crossings and potentiated AMPH effects on stereotyped behavior (Figueira et al., 2015) while gabapentin prevented the AMPH-induced stereotyped behavior without effects on the number of crossings (Ceretta et al., 2016). In fact, data from literature suggest that occurs a behavioral sensitization to AMPH in a differential manner leading to different responses observed for both stereotypy and hyperlocomotion despite of both behavior to be related to same brain regions (Robinson and Becker, 1986).

As previously mentioned, MAO is an enzyme that catalyzes the oxidative deamination of monoamines. It is present in two isoforms (MAO-A and MAO-B) in the most mammalian tissues (Shih et al., 1999; Tipton et al., 2012). MAO-A has high affinity for the hydroxylated amines as serotonin, norepinephrine, and by the inhibitor clorgyline. Whereas MAO-B has high affinity for the non-hydroxylated amines as beta-phenylethylamine, benzylamine and by the inhibitors selegiline, pargyline, rasagiline and low concentrations of selegiline. The amines DA, tyramine, epinephrine and tryptamine have affinity for both isoforms (Finberg and Rabey, 2016; Youdim et al., 2006; Youdim and Bakhle, 2006). Regarding degradation of DA, it can vary in relation to the specie and the tissue under consideration. In the rodent striatum, DA is metabolized preferentially by MAO-A isoform under basal conditions and, by both (MAO-A and MAO-B) in high concentrations (Fornai et al., 1999; Youdim and Bakhle, 2006).

In vitro studies demonstrate that Kava extract inhibits preferentially MAO-B (Prinsloo et al., 2019; Uebelhack et al., 1998); however, the effects of Kava extract on MAO activity *in vivo* are scarce. As AMPH also inhibits the activity of both isoforms of MAO (Faraone, 2018; Miller et al., 1980; Robinson, 1985) we investigated the possible effects on both isoforms since a combined effect of Kava extract and AMPH could be involved in the behavioral changes found in this study. However, besides an effect of Kava extract on MAO-A activity in cortex and MAO-B activity in hippocampus and striatum, no statistical correlation was found between the activity of MAO and locomotion and stereotyped behavior (data not shown). Since the *in vitro* binding of Kava extract (Uebelhack et al., 1998) and AMPH (Miller et al., 1980) are reversible, the binding with the enzyme is highly dynamic which could explain the lack of effects on MAO activity in most of evaluated tissues.

Additionally, it was performed the evaluation of social interaction and Y maze (spatial working memory). As expected, AMPH does not produce alterations in both tests, which is in agreement with other studies (Featherstone et al., 2007; Jones et al., 2011; Kameda et al., 2013; Kane et al., 2011; Lieberman et al., 1987). In Y maze test, there was no significant effects of Kava extract/AMPH on the percentage of correct alternations. However, a significant effect of Kava extract increasing the number of entries into the arms was observed which could be related to the increased locomotor activity present in the animals in the open field test.

5. Conclusion

Taken together, the present study demonstrated that the Kava extract prevented the appearance of stereotyped behavior induced by AMPH in mice, suggesting a potential therapeutic in psychotic symptoms. Furthermore, Kava extract decreases the stereotyped behavior at the same dose which could help to alleviate anxiety symptoms found in patients.

Co-authors contribution

De Freitas, C. M.; Ceretta, A. P. C.; Barbosa, C. P.; Reis, E. de M.: Behavioral and biochemical analysis. Scussel, R.; Corneo, E. S.; Machado-de-Avila, R. A.: Biochemical analysis. Boligon, A: carried out HPLC analysis. Krum, B.: Fachinetto, R.: investigation and

writing–original draft preparation.

Declaration of competing interest

The authors declare that there are no conflicts of interest associated with this study.

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