SHORT COMMUNICATION

Acute oral toxicity, antinociceptive and antimicrobial activities of kava dried extracts and synthetic kavain

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*ABSTRACT*

Piper methysticum G. Forst, popularly known as kava, is a traditional medicinal plant widely used for the treatment of anxiety and insomnia. The aim of this study was to investigate new therapeutic applications of this plant. Nociceptive response induced by heat (hot-plate) was used as pain model. Susceptibility of different strains to kava ethanolic dried extracts was evaluated by broth microdilution method. Acute oral toxicity was performed according to Organisation for Economic Cooperation and Development (OECD) guideline. Administration of kava dried extracts and kavain inhibited the nociceptive response in the hot-plate model and did not affect the time mice spent in the rota-rod apparatus. The samples showed no significant antibacterial activity, however slight antifungal activity was verified. The extracts may be considered of low oral acute toxicity. Kava extracts exhibited promising antinociceptive activity in model of nociceptive pain, which should be deeper explored as a new therapeutic application of kava.

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1. Introduction

Kava (Piper methysticum G. Forst) is a native plant from South Pacific Islands, where beverages and extracts of roots and rhizomes were consumed due to its psychotropic, sedative and muscle relaxant properties. Currently, the commercial preparations of kava extracts are widely used to treat mild to moderate states of anxiety, insomnia and muscle pain (Singh 2004; Siméoni and Lebot 2014; Bian et al., 2020). These activities are directly associated with the psychoactive constituents of kava, known as kavalactones, a mixture of structurally related lipophilic lactones with an \( \alpha \)-pyrone skeleton. The six major kavalactones are kavain, dehydrokavain, demethoxyyangonin, yangonin, dihydromethysticin and methysticin (Bilia et al. 2004; Ferreira et al. 2021). Beyond the well established use as sedative and relaxant, other therapeutic effects are also attributed to kava extracts, such as anticancer, anticonvulsant, antithrombosis, antimicrobial, analgesic and for attenuation of menopausal symptoms (Jamieson and Duffield 1990; Uebelhack et al. 1998; Côté et al. 2004; Pereira et al. 2015; Celentano et al. 2020). However, some of these reported studies are still incipient and the additional claimed properties of the extracts needs to be deeper investigated for a better understanding and screening of the therapeutic potential of kava. Within this context, the search for new antimicrobial agents is essential, considering the significant increase of bacterial and fungal resistance. The number of studies relating the use of natural products with antimicrobial activity has significantly increased in recent years (Gyawali and Ibrahim 2014; Moloney 2016). Equally important is the development of novel agents and treatments for management of pain. Although the extensive knowledge about peripheral and central sensitisation mechanisms, many drugs exhibit low efficacy or unacceptable side effects (Yekkirala et al. 2017).
On the other hand, concerns over hepatotoxicity have led to its withdrawal or restrictions in many countries (Sarris et al. 2011). Further studies demonstrated that flavokavain B, a chalcone from kava root, is highly cytotoxic to human hepatoma HepG2 cells (Zhou et al. 2010). An important point to assure the safety of kava-based therapy is the differentiation between so-called two-day kava and noble kava varieties. Kava noble cultivars have high content of kavalactones associated to low levels of flavokavain B, whereas the ratio flavokavain B/kavalactones is higher in two-days kava (Lebot et al. 2014). Nevertheless, the composition and the concentration of the herb constituents may vary due to factors such as harvesting, drying, storage and processing, so that the assessment of acute oral toxicity of kava-based products marketed nowadays is an important tool to assure the safety of the treatment.

The widely commercialisation of herbal products based on kava evidences the need of a deeper investigation about its therapeutic potential, searching for new pharmacological application for this traditional plant. Hence, the aim of this study was to evaluate the antimicrobial and antinociceptive activities of kava dried extracts, as well as the acute oral toxicity in murine model.

2 Results and discussion

The exposure of the animals to the noxious heat induced a nociceptive response, which was characterised by licking the hind paws or by jumping off the hot plate. Preadministration (2 h) of extracts from sample 1, 5 and 8 (150 mg/kg; p.o), kavain (7 mg/kg; p.o) or dipyrene (500 mg/kg; p.o; positive control) increased the animal’s latency to exhibit the nociceptive behavior ($p < 0.05$) (Figure 1S). To validate the results of antinociceptive activity, we investigated the effects induced by extracts and kavain on the motor coordination of mice. The time mice spent on the rotating rod was not altered 2 h after administration of extract from sample 1, 5 and 8 (150 mg/kg; p.o) or kavain (7 mg/kg; p.o). The positive control, phenobarbital (50 mg/kg, p.o.), markedly inhibited the performance of the animals in the rota-rod apparatus ($p < 0.05$) (Table 1S). We observed that kava dried extracts, as well as kavain, markedly increased the latency for the nociceptive response induced by heat in the hot-plate model. As the kava dried extracts used in the present study did not change the time mice spent on the rotating rod, it is unlikely that inhibition of the nociceptive behavior is due to a confounding effect on motor coordination or muscle tone. Kava extracts activity in this experimental model suggests an inhibitory effect on the nociceptive processing in the central nervous system and/or on the nociceptors activation by the thermal stimulus (Julius and Basbaum 2001; Vriens et al. 2011; Jang et al. 2020). Decrease in nociceptive response was also observed by Jamieson and Duffield in an animal model of abdominal constriction induced by acetic acid injection, after oral administration of kava resin and aqueous extract (Jamieson and Duffield 1990). This previous work demonstrated that the analgesia from kava extracts occurs via non-opiate pathway, since the antinociceptive effect was not reversed by administration of naloxone, an opioidergic antagonist (Jamieson and Duffield 1990). Since kavain also showed statistical difference regarding the vehicle latency time, the antinociceptive activity of the extract may be related to the presence of this kavalactone. On the other hand, we found no
correlation between antinociceptive activity of the extracts and the content of kavalactones or kavain. Despite the variation in kavain and total kavalactones contents between the kava extracts (Table 3S), no significant difference was evidenced in the antinociceptive activity of these samples.

Evaluation of the antimicrobial study indicated that neither the extract samples nor kavain showed antibacterial activity against the bacteria strains evaluated in this study (MIC > 512 μg/mL). Except for Cryptococcus spp, no evaluated fungi strain had their growth inhibited by the extracts and kavain. Regarding antifungal activity against Cryptococcus spp, Table 2S shows MIC values determined for each tested sample against different strains. Samples 1 and 2 were not able to inhibit fungi growth, while extract 4 was the only sample with MIC values of 256 μg/mL for both C. gatti strains. The assayed samples did not present antibacterial activity against the bacteria evaluated in this study, which is consistent with Locher findings (Locher et al. 1995). Studies developed by Pereira evidenced antibacterial activity of kava extracts against S. aureus and E. coli (Pereira et al. 2015). However, Pereira evaluated hydroalcoholic extracts but not ethanolic samples and the difference in the extraction solvent can directly influence the qualitative and quantitative composition of the active markers of the extracts and consequently its biological activities (Xuan et al. 2008). Antifungal activity was evidenced only against Cryptococcus strains. Even though studies about antifungal activity of kava can be found, these refer to aqueous extracts of kava (Guérin and Réveillère 1984; Locher et al. 1995) and, similarly, the difference in the extraction solvent can directly influence the extract antimicrobial activities. Overall, lower MIC values were observed for C. gatti R265 when compared to C. gatti ATCC 24065, even though R265 has better defined virulence factors and higher MIC would be expected. Interestingly, previous studies with C. gatti strains have shown that a more virulent strain may also be more susceptible to antifungal treatment (Santos et al. 2014). Since no antifungal activity of kavain was observed, the growth inhibition promoted by the extracts probably was not related to this kavalactone.

It was observed that the administered dose (2000 mg/kg) of extract samples 1 and 4 did not cause death to the animals during the two levels of the study. Due to this absence of mortality, kava extracts evaluated in this study can be classified in category 5 or unclassified by the Globally Harmonised System (GHS) criterion, meaning that the extracts have no identified toxicity up to 2000 mg/kg. The extract treatment did not influence the body mass gain and variation in relation to the control group, showing no statistical difference (p > 0.05). Regarding the feed intake and the relative weight of the kidneys and livers of the animals, no significant difference was observed between the values obtained in control and treated groups after 14 days (p > 0.05) (Figure 2S). Histological analysis evidenced no apparent changes in any of the evaluated kidney and liver. All slides presented histology similar to each other and according to the morphological characteristics of the respective organ (Figure 3S). The nonappearance of moderate or severe behavioral alterations added to the absence of apparent changes during histological analysis of the kidney and liver after oral administration of kava extracts indicates that the samples are classified as category 5 and may be considered of low oral acute toxicity. Thus, kava extracts presented no identified toxicity up to 2000 mg/kg. According to previous data, LD50 observed after oral administration
of acetonic kava extract (70% kavalactones) in mice and rats were close to 1500 mg/kg. The higher percentage of kavalactones in the acetonic extract may be directly related to the obtained lower LD$_{50}$ value (WHO 2004; EMA 2016). The results are consistent with those previously reported, in which the administration of aqueous, methanolic and ethanolic extracts in animals did not promote impairment in liver function (Singh and Devkota 2003; Sorrentino et al. 2006; Lim et al. 2007; Petersen et al., 2019).

3 Conclusion

In conclusion, it was explored relevant pharmacological activities of kava dried extracts, usually employed as raw material for kava-based formulations. To our knowledge, it was the first time that the antinociceptive activity of kava dried extract has been demonstrated in a murine model with response induced by heat. The antinociceptive effect of kava extract was highlighted and further studies are needed to investigate the phytoconstituents associated to this effect, as well as to determine the pathway by which the observed analgesia occurs. Altogether, the results indicate that kava dried extracts should be further investigated aiming to their use for other conditions, as well as in the treatment of patients with different painful conditions.

Disclosure statement

No potential conflict of interest was reported by the authors.

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