Evaluation of Anti-Aggressive Activity of *Kyllinga brevifolia* in Rodent Model of Aggression

M.C. Hellión-Ibarrola*, M.L. Kennedy, M.A. Campuzano, Y. Montalbetti, O.Y. Heinichen and D.A. Ibarrola

Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Asunción, Campus UNA, 2169, San Lorenzo, Paraguay

Abstract: *Kyllinga brevifolia* Rottb, Cyperaceae, rhizomes are used in the Paraguayan folk medicine as sedative and tonic for nervous system. The aim of this study was to determine the anti-aggressive activity of hydro-ethanolic (rhizomes) extract (CEKb), hexane (KbF-hex), chloroform (KbF-chlo), ethyl acetate (KbF-ethyl-ac) and aqueous (KbF-aq) fractions of *K. brevifolia* on the aggressive behavior assessed in the isolation-induced aggression model in male mice. The effect on aggressive behavior was observed during a ten minute resident-intruder confrontation. Oral doses of CEKb (10 and 100 mg/kg; *p*< 0.05), KbF-hex (0.1, *p*<0.01; 1, *p*< 0.001 and 10 mg/kg, *p*< 0.01)) and KbF-ethyl-ac (0.1, *p*<0.01; 1, *p*< 0.05 and 10 mg/kg, *p*< 0.05) from *K. brevifolia* reduced fighting time of isolation-induced aggressive behavior, respectively, in male mice. In conclusion, our results make evident that CEKb, Hex Kb and KbF-ethyl-ac obtained from *K. brevifolia* possesses anti aggressive-like property in mice. These finding give further support to the traditional use of *Kyllinga brevifolia* as sedative and tonic in Paraguayan folk medicine. Taken together, these findings suggest that *Kyllinga brevifolia* exhibits a general anti-offensive aggressive activity and may be relevant in the treatment of reactive aggression in humans.

Keywords: *Kyllinga brevifolia*, anti-aggressive, fighting time, isolation-induced aggressive behavior.

1. INTRODUCTION

Social conflict and intra-specific aggression are key aspects of natural animal behaviour and have been shown to be a biologically relevant mean of examining central neurochemical functions. A number of ways have been developed to induce aggression in mice. Administration of anxiolytic agents has been found to be useful in the suppression of aggressive behaviour in animal models [1].

Data from literature related to the effects of drugs on isolation-induced aggression in mice are generally agreed, in spite of the use of different methodologies (e.g., induction techniques, testing conditions, scoring systems). Fighting behaviour in isolated mice has been shown to be selectively antagonized by antidepressants, neuroleptics, anticholinergics, antiserotonergics and antihistaminic drugs. However, aggression is not selectively antagonized by anxiolytics, muscle relaxants, anticonvulsants, sedatives and hypnotics [2, 3].

According to Weinshenker and Siegel, (2002) aggression is a deliberate series of actions that lead to harm or injury to another organism and thus constituting a major public health concern across the globe. In clinical settings, aggression has been classified into two more specific subtypes, such as proactive and reactive aggression. Proactive aggression is overcontrolled, planned, predatory and driven by reward contingencies, whereas reactive aggression is generally characterized by an overaroused and impulsive response to a perceived threatening stimulus, with a single goal of reducing or eliminating the perceived threat [4]. Offensive aggression in animals possesses many of the characteristic features of reactive aggression in human beings including impulsive responses and neurochemical abnormalities [5]. The use of animal models of aggression affords the possibility of assessing the effects of drugs on specific types of aggression.

Aggression is caused by a heterogeneous mixture of social, psychologic and biological factors. Involvement of y-aminobutyric acid (GABA)-ergic neurotransmission in the neurobiology of aggressive behaviour has often been reported. It has been suggested that agents acting on the GABA<sub>A</sub> receptor complex, could be biological modulators of aggression. Si-Wu-Tang, a Sino-Japanese traditional prescription (a mixture of underground organs of *Rehmanniae* sp, *Paeoniae alba*, *Angelica sinensis* and *Ligusticum* sp), had demonstrated efficacy against isolation-induced aggressive behaviour in mice [6]. It has also been reported that small amounts of alcohol, the neuroactive steroid 3α, 5α-tetrahydroprogesteroneand benzodia-zepines may heighten aggression in humans as well as in animals, whereas higher doses reduce aggression [7].

*Address correspondence to this author at the Facultad de Ciencias Químicas, Universidad Nacional de Asunción, Campus UNA, 2169, San Lorenzo, Paraguay; Tel: + 595 21 585562; Fax: + 595 21 585564; E-mail: chellion@qui.una.py*
Kyllinga brevifolia Rottb. (Cyperaceae) (Cyperus brevifolius (Rottb) Hassk) (kapi-i katí) leaves and rhizomes are used in Paraguayan traditional medicine as sedative and tonic, as well as carminative and digestive among others [8-10]. We have previously investigated the effect of K. brevifolia rhizomes on intestinal transit, sleeping time induced by barbiturate, acute toxicity [11], and the anxiolytic-like effect in mice [12]. Previous researches revealed the presence of flavonoids on K. brevifolia rhizomes [13].

The present study was undertaken to determine the influence of the hydro-ethanolic (rhizomes) extract and fractions of K. brevifolia on the aggressive behavior in male mice, using the isolation-induced aggression method.

2- MATERIALS AND METHODS

2.1. Plant Material and Extraction

Kyllinga brevifolia Rottb. (Cyperus brevifolius (Rottb) Endl. ex Hassk), Cyperaceae, locally known as kapi-i katí, were collected in Paraguarí Department, Paraguay, in February 2011 and identified by Isabel Basualdo, a voucher specimen is deposited at the herbarium of Facultad de Ciencias Químicas, Universidad Nacional de Asunción under the number Basualdo 2900. Fresh rhizomes were air-dried and ground, yielding 459.5 g of powder. The powder was extracted with a mixture of ethanol:water (70:30) by a conventional reflux method, filtered and evaporated under reduced pressure. The concentrated extract was frozen and finally freeze-dried, yielding 31g of lyophilized rhizome extract (CEKb). 5.2g of CEKb was suspended in 500 mL of deionized water and successively extracted with n-hexane, chloroform and ethyl acetate (3 x 500 mL each). n-Hexane (KbF-hex) and chloroform (KbF-chlo) fractions were dried with anhydrous sodium sulphate, filtered and evaporated under reduced pressure at 40 ºC. Ethyl acetate fraction (KbF-ethyl-ac) was concentrated under reduced pressure and the aqueous solution (KbF-aq) was freeze-dried.

2.2. Animals

Adult Swiss albino male mice weighing 20-30 g were used in this study. They were fed with balanced pellet diet and water ad libitum, housed in plastic cage at a constant room temperature (23-25°C), with 12:12 h light-dark cycle, in humidity controlled environment (50-60%). All experiments were conducted in accordance with international standards of animal welfare and experimental protocols were approved by the Bioethical Committee of the Facultad de Ciencias Químicas (FCQ-2004/01). The minimum number of animals and the duration of observation required to obtain consistent data were used, each animal was used once. Behavioral experiments were conducted from 09:00 AM to 2:00 PM.

2.3. Drugs

Diazepam (Valium) was from Roche Pharmaceutical Co., Ltd. (Argentine), sodium sulphate and sodium chloride are obtained from Sigma Chemical Company (St. Louis, MO, USA), ethanol, chloroform, ethyl acetate, n-hexane, tween 80 and propylenglycol were purchased locally, all solvents were distilled before use.

2.4. Isolation-Induced Aggressive Behavior

Male mice (20-30 g) were randomly distributed into groups of ten animals per treatments and then were individually isolated and fed in their cages for a 7-weeks period as described by Watanabe [6] with minor modifications. After a 4-weeks isolation period a male intruder mouse of similar size and weight was placed (once a week) into the cage of the isolated mouse for strengthening his aggressive behaviour. After aggressive behaviour was installed, isolated mice were treated in individual session with: (A) vehicle (0.1 mL/10 g body weight p.o), (B) doses of 1, 10, 100 and 1000 mg/kg p.o., of CEKb, C) doses of 0.1, 1 and 10 mg/kg, p.o., of KbF-chlo, KbF-aq, KbF-hex and of Kb-ethyl-ac fractions and (D) dose of 10 mg/kg p.o., of diazepam. After 60 min of treatment, a non-isolated male mouse (intruder) was placed into the home cage of the isolated one and the duration of biting attacks and/or wrestling was measured for 10 min [1]. Analysis of the data’s were performed according to the initial assigned groups of treatments.

2.5. Statistical Analysis

Results are expressed as mean ± S.D. the data were analyzed using GraphPad Prism 5.0 software (GraphPad Software, Inc. CA, USA) by Dunn’s Multiple Comparison test after Kruskal-Wallis non-parametric ANOVA. A level of p<0.05 was considered as statistically significant.

3. RESULTS AND DISCUSSION

We have previously reported that Kyllinga brevifolia, stimulate intestinal transit, increases sleeping time
induced by barbiturate and exert an anxiolytic-like activity in mice [11, 12].

As depicted in Figure 1, the oral administration of 10.0 and 100.0 mg/kg of CEKb, induced a significant reduction (p<0.05) in the aggressive behavior (fighting time) of isolated mouse. In addition, oral administration of 0.1, 1.0 and 10.0 mg/kg of KbF-hex (p<0.01; p<0.001; p<0.01) and 0.1, 1.0 and 10.0 mg/kg of KbF-ethyl-ac (p<0.01; p< 0.05; p< 0.05) fractions provoked a significant reduction in aggressive behavioral of isolated mouse (Figures 2 and 3). The KbF-chlo and KbF-aq from CEKb did not modify the aggressive behavior of mice (Figure 4). According to these data, CEKb exhibits anti-aggressive-like effect, because aggressive behavior is reduced in a similar pattern as diazepam (10 mg/kg). The bioguided fractionation demonstrated that the same activity remains in hexane and ethyl acetate fractions, since a significant reduction in fighting time of isolated mouse was observed after oral treatment with KbF-hex and KbF-ethyl-ac respectively.
According to literature flavonoids glycosides are the major constituents of *K. brevifolia* rhizomes [13], but in this case the compounds to which anti aggressive activity can be attributed were not identified yet. Furthermore, due to several mechanisms are known to affect aggression in both non specific (sedation, motor disturbance, psychostimulation) and specific (5-HT<sub>1A</sub>, 5-HT<sub>1B</sub> agonist) ways [3], at this stage, it is not possible to predict the mechanism of anti aggressive effect of *K. brevifolia*.

In the isolation-induced aggressiveness test on male mice, it was found that the anti aggressive effects of benzodiazepines are both, related (bromazepam, diazepam, nitrazepam, temazepam, and desmethyl-diazepam) and unrelated (chlorodiazepoxide, oxazepam and medazepam) to their possible muscle-relaxing effect [14]. In summary, our results give further support to the traditional use of *Kyllinga brevifolia* in Paraguayan folk medicine. These findings may provide important leads for the development of potent and selective anxiolytic agents which could also be devoid of the possible disadvantage arising from their oral uptake.

On the other hand, the term aggression is widely employed to indicate various patterns of psychological or sociological behavior resulting from pathological, biochemical or physiological alteration of central nervous system which disturbed emotional regulation [17]. In addition, it was showed that social isolation-induced aggression could potentiate anxiety and depressive-like behaviors in isolated male mice subjected to unpredictable chronic mild stress [16]. There are many psychiatric disorders such as schizophrenia and Alzheimer’s disease which show close association with aggression [17]. In conclusion, our results make evident, with some limitations, that *K. brevifolia* (CEKb, Hex Kb and KkB-ethyl-ac) possesses anti aggressive-like property in mice and could be a potential medicine for the treatment of aggression condition in humans. However, further studies using other methods and measuring several parameters are necessary to confirm, extend and clarify the underlying mechanism of these effects. Somehow, the findings presented here are relevant because they validate the folk uses of *K. brevifolia*, an important medicinal plant used in Paraguay.

**ACKNOWLEDGEMENTS**

This work was performed under the financial support of Fondo Central de Investigaciones de la Universidad Nacional de Asunción, la Facultad de Ciencias Químicas and SECAB-CYTED (Secretaría Ejecutiva del Convenio Andrés Bello-programa CYTED CyT N°1701/K1 2002). The present work was made within the Doctoral program in Pharmaceutical Sciences of the Faculty of Chemical Sciences. We also want to thank to Nelson L. Álvarez and Esteban A. Ferro for technical support in the fractionation of the crude extract, and Rosa Degen for Botanical identification.

**REFERENCES**


Received on 19-09-2013 Accepted on 02-10-2013 Published on 02-04-2014

DOI: http://dx.doi.org/10.14205/2312-3710.2014.01.01.1

© 2014 Hellión-Ibarrola et al.; Licensee Pharma Publisher. This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.