South African Journal of Botany 94 (2014) 33–39

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

# Long-term cardiovascular autonomic responses to aqueous ethanolic extract of *Boophone disticha* bulb in early maternally separated BALB/c mice

William Pote [a,*], Dexter Tagwireyi [b], Herbert M. Chinyanga [c], Colin Musara [a], Davies M. Pfukenyi [d], Pilani Nkomozepi [e], Louis L. Gadaga [b], George Nyandoro [a], Jephat Chifamba [c]

[a] Preclinical Veterinary Studies Department, Faculty of Veterinary Science, University of Zimbabwe, Zimbabwe
[b] Drug and Toxicology Information Service, School of Pharmacy, College of Health Sciences, University of Zimbabwe, Zimbabwe
[c] Department of Physiology, College of Health Sciences, University of Zimbabwe, Zimbabwe
[d] Clinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe, Zimbabwe
[e] Division of Neuroscience, University of Witwatersrand, South Africa

ARTICLE INFO

*Article history:*
Received 10 December 2013
Received in revised form 16 April 2014
Accepted 22 April 2014
Available online 19 June 2014

Edited by JJ Nair

*Keywords:*
*B. disticha*
Antihypertensive activity
Autonomic response
Cardiovascular comorbid disorders
Anxiety disorders

ABSTRACT

*Background: Boophone disticha* is commonly used in southern Africa for the management of mental-related illnesses. Recently, it was shown to reduce blood pressure (BP) in maternally separated mice immediately after withdrawal of treatment. However, the long-term cardiovascular effects and the underlying mechanisms are still illusive. Based on the reputed traditional use of the plant for anxiety and stress-related disorders, the aqueous-ethanolic extract of *B. disticha* was screened for its long-term effects on the cardiovascular and autonomic responses to repeated acute stressors in adult early maternally separated BALB/c mice.
*Methods:* Five groups (n = 6 each) of adult BALB/c mice subjected to early maternal separation (MS) were given six daily oral doses of vehicle (normal saline); low, medium and high doses of *B. disticha* (10, 25 and 40 mg/kg body weight, respectively); and 1 mg/kg body weight diazepam during adulthood. The control (un-separated) group (n = 6) received vehicle treatment. Cardiovascular parameters (BP and heart rate (HR)) were recorded using non-invasive tail-cuff methods on post-treatment days (PTDs) 9 and 30 to compare short-term and long-term effects of the plant extract, respectively. Autonomic responses were measured by estimating BP variability (BPV) and HR variability (HRV).
*Results:* Early maternal separation significantly increased systolic BP (SBP), and decreased HR on PTD9 while raising BPV on PTD30 when compared to control un-separated mice ($p < 0.05$). *B. disticha* at low dose significantly reduced short-term SBP and mean arterial pressure (MAP), while medium dose reduced long-term diastolic BP (DBP) and MAP in maternally separated mice when compared to vehicle and diazepam ($p < 0.05$). High dose significantly decreased SBP and MAP at both occasions ($p < 0.05$).
*Conclusions:* The current results have led to the identification of long-term antihypertensive-like activity of the aqueous ethanolic extract of *B. disticha* which was found to last for several weeks after withdrawal of treatment.


## 1. Introduction

*Boophone disticha* (L.f.) Herb. (Amaryllidaceae) known as 'sore-eye flower' ('munzepete' in Shona and 'ingcotho' in IsiNdebele), is used for medicinal purposes among the indigenous people of southern Africa. It is used for the treatment of several mental-related conditions such as epilepsy, seizures, psychosis, anxiety, depression and cognitive disorders (De Smet, 1996; Gelfand and Mitchell, 1952; Gelfand et al., 1985; Gomes et al., 2009; Hutchings et al., 1996; Nair and Van Staden, 2014; Stafford et al., 2008; Steenkamp, 2005). Although, the mechanism underlying the effects of the plant is largely unknown, *in vivo* studies done on the crude extract showed that the plant has potential antidepressant (Chingombe et al., 2010; Pedersen et al., 2008), anxiolytic (Chuma et al., 2010; Musarira et al., 2011), nootropic (Gadaga, 2012) and antihypertensive (Pote et al., 2013) activities in animal models.

Anxiety and depression disorders are the most prevalent psychiatric conditions. These conditions are commonly undertreated because they occur along with other mental or physical illnesses, mostly comorbid cardiovascular disorders, that mask their symptoms causing socio-

*Abbreviations:* AMS, acute mixing stress; BP, blood pressure; BPV, blood pressure variability; BW, body weight; DBP, diastolic blood pressure; DZP, diazepam; HDBD, high dose *B. disticha*; HR, heart rate; HRV, heart rate variability; LDBD, low dose *Boophone disticha*; MAP, mean arterial pressure; MDBD, medium dose *B. disticha*; MS, maternal separation; PND, postnatal day; PTD, post-treatment day; SBP, systolic blood pressure; Veh, vehicle.

* Corresponding author at: PO Box MP167, Mount Pleasant, Harare, Zimbabwe. Tel.: +263 773234621, +263 736566176; fax: +263 4333678.
*E-mail addresses:* w.pote@yahoo.com, potewilliam@gmail.com, wpote@medic.uz.ac.zw (W. Pote).

http://dx.doi.org/10.1016/j.sajb.2014.04.012
0254-6299/

economic burden and personal distress (Belzung and Griebel, 2001; Sarris and Kavanagh, 2009). Current treatments are less than satisfactory; for example, a considerable proportion of patients are non-responsive to first-line treatment, the onset of action is delayed and the drugs can induce side effects which significantly impair compliance (Sarris and Kavanagh, 2009). In addition, these medications are beneficial to only 65% of the patients; hence, most people with severe depression and anxiety attacks use complementary and alternative medicine to treat these conditions (Sarris and Kavanagh, 2009). Since anxiety significantly increases morbidity and mortality in heart diseases (Belzung and Griebel, 2001), successful treatment for psychiatric disorders should aim to correct the abnormalities of central cardiovascular autonomic regulation (Cohen and Benjamin, 2006; Depino and Gross, 2007; Igosheva et al., 2004; Volkmar et al., 2005).

Most recently, a study done by Pote et al. (2013) found that *B. disticha* extract (10 to 40 mg/kg body weight (BW)), significantly reduced systolic BP (SBP), diastolic BP (DBP) and mean arterial pressure (MAP) in maternally separated (MS) mice immediately after withdrawal of treatment. *B. disticha* may therefore be utilized to develop a drug for treatment of comorbid cardiovascular disorders that are frequently associated with anxiety and mood disorders. However, the low response rate to treatment makes it very difficult to prove on short-term basis that the plant has anxiolytic, antidepressant or antihypertensive efficacy. Therefore, the present study was set to explore the long-term pharmacological effects of the aqueous ethanolic extract of *B. disticha* on blood pressure (BP), heart rate (HR), BP variability (BPV) and HR variability (HRV) in a maternal separation animal model of anxiety disorder.

## 2. Materials and methods

The experimental protocols, care and handling of animals used in this study were in accordance with international guidelines (European Community guidelines, EEC Directive of 1986; 86/609/EEC) on the use and care of laboratory animals and were approved by the Division of Veterinary Services, Zimbabwe (Pote et al., 2013).

### 2.1. Plant materials, extraction and qualitative analysis of alkaloids

Bulbs of *B. disticha* were harvested, authenticated, and dried and the aqueous ethanolic (70% v/v) extract was prepared as described previously (Gadaga et al., 2011; Pote et al., 2013). Chromatographic analysis (Zulu et al., 2011) confirmed the presence of isoquinoline alkaloids reported in previous studies (Adewusi et al., 2012; Cheesman et al., 2012; Hauth and Stauffacher, 1961; Neergaard et al., 2009; Sandager et al., 2005; Steenkamp, 2005).

### 2.2. Animals and housing conditions

Pregnant BALB/c mice, obtained from the Central Veterinary Laboratory Breeding Unit in The Ministry of Agriculture (Zimbabwe), were housed in the Animal Holding facilities at the University of Zimbabwe (UZ) under conditions previously described by Pote et al. (2013).

### 2.3. Experimental protocols

Control pups (un-separated group) were not separated from their dams during the deprivation period while the treatment pups were maternally separated. The procedures for maternal separation (MS) were as described earlier by Pote et al. (2013). The study animals were weaned, caged, grouped and given treatments from post-natal day (PND) 71–76 as reported previously (Pote et al., 2013). Briefly, the control group (Group A) comprised of six mice (three males and three females) randomly selected from an un-separated litter. For the treatment groups, 30 mice (15 males and 15 females) were selected randomly from a maternally separated colony. These were randomly assigned to five treatment groups: B, C, D, E, and F ($n = 6$ each; with 3 males and 3 females housed in separate cages, Fig. 1). Groups A (control + Veh) and B (MS + Veh) were given a vehicle (normal saline). Groups C (MS + LDBD), D (MS + MDBD) and E (MS + HDBD) received low (10 mg/kg body weight (BW)), medium (25 mg/kg BW) and high (40 mg/kg BW) doses of *B. disticha*, respectively; while Group F (MS + DZP) received 1 mg/kg BW diazepam (Fig. 1).

#### 2.3.1. Cardiovascular responses to acute mixing stress

The present study repeated the procedures of acute mixing stress (AMS) and restraint stress described recently by Pote et al. (2013) on post-treatment day 9 (PTD9) and PTD30 (*i.e.* PND85 and PND106, respectively). Blood pressure (systolic blood pressure (SBP), diastolic BP (DBP), mean arterial pressure (MAP)) and heart rate (HR) were recorded for 5 min between 09.00 and 15.00 h on PTD9 (short-term response) and PTD30 (long-term response). A non-invasive tail-cuff system (BIOPAC® System Inc., CA) was used to monitor all the cardiovascular parameters (Pote et al., 2013).

#### 2.3.2. Autonomic reactivity

The standard deviation of the MAP ($SD_{MAP}$) calculated for every segment (24 s) of the recording period was used as a measure of blood pressure variability (BPV) (Igosheva et al., 2004). Standard deviation of heart rate ($SD_{HR}$) calculated from every heart beat, recorded on the interval between the SBP and DBP, was used as a measure of heart rate variability (HRV).

### 2.4. Statistical analysis

Data was initially captured with Excel where it was sorted and then exported to statistical package software (SPSS® version 16.0) for further analysis. Comparisons were done on treatment groups using multivariate multiple comparison analysis and independent Student's t-test. Changes in cardiovascular parameters were calculated by subtracting values recorded on PTD9 from PTD30 readings and the mean changes were analyzed using independent t-test and one-way ANOVA with multiple comparisons across all test groups. The normal mice (Group A; Control + Veh) was first compared to Group B (MS + Veh; Fig. 1) on PTD9 and PTD30 to determine how habituation to repeated acute mixing stress would affect the cardiovascular and autonomic reactivity. MS mice treated with three doses of *B. disticha* extract (low, medium and high doses: Groups C, D and E), were compared to the vehicle (Group B) and diazepam (Group F; Fig. 1). Comparisons were also made on normal un-separated mice that received vehicle (Group A) and all MS treated mice Groups C–F (Fig. 1) to find which treatment would retain and maintain the animal health towards the normal range on PTD9 and PTD30. All data were expressed as mean difference ± standard error (S.E.) with a significance level of $p < 0.05$.

## 3. Results

MS mice had a significantly higher SBP when compared to control mice on PTD9 with a mean difference of 4.6 ± 1.8 millimeter mercury (mm Hg, $p < 0.02$) but this effect was absent on PTD30 ($p > 0.05$). DBP, MAP and changes in BP recorded over the entire test period were comparable between the two groups ($p > 0.05$) (Fig. 2). When early maternally separated mice were compared to the control, initially on PTD9 they exhibited lower HR (mean difference 53.7 ± 16.5 beats per minute (bpm); $p < 0.001$), but the difference disappeared on PTD30 (mean difference 11.8 ± 13.9 bpm; $p > 0.05$). The change in HR over the testing period was non-significantly higher in the former group ($p = 0.091$).