Cardiotoxic effects of pavetamine extracted from *Pavetta harborii* in the rat

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ABSTRACT

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Previous studies have shown that crude extracts from *Pavetta harborii* as well as dried plant material have cardiotoxic effects on rats and sheep that can lead to heart failure. The active component has since been isolated and identified. This substance has been named pavetamine. The aim of this study was to determine whether pavetamine has cardiotoxic effects similar to those seen in previous reports, when administered to rats intraperitoneally. Sprague Dawley rats received two doses, initially 4 mg/kg and then 3 mg/kg pavetamine respectively and were monitored for 35 days before cardiodynamic parameters were measured by inserting a fluid-filled catheter into the left ventricle via the right carotid artery. These values were compared to those of control rats that had received only saline. Pavetamine significantly reduced systolic function and body mass in the treated rats, which indicates that it has the potential to induce heart failure in this animal model.

Keywords: Cardiodynamics, cardiotoxic, pavetamine, *Pavetta harborii*, rat

INTRODUCTION

Six species of the plant family Rubiaceae, viz. *Pachystigma latifolium, Pachystigma pygmaeum, Pachystigma thamnus, Pavetta harborii, Pavetta schumanniana* and *Fagodia homblei* cause gousiekte, a syndrome characterized by heart failure and sudden death, when ingested by ruminants (Theiler, Du Toit & Mitchell 1923; Pretorius & Terblanche 1967; Kellerman, Coetzer, Naudé & Botha 2005). A number of cardiodynamic changes (Pretorius & Terblanche 1967) as well as myocardial lesions that consist of a loss of myofilaments, replacement of myocytes with collagenous tissue, lengthening of sarcomeres and cardiac dilatation (Newsholme & Coetzer 1984) have been reported. Apart from the economic impact on farming with domestic ruminants the plant has attracted the attention of researchers for its possible use as a model for studying heart failure.

One model that was successfully used in sheep was that produced by addition of dried *Pachystigma pygmaeum* leaves to a normal diet (Schutte & Du Plooy 1990). A later study also succeeded in inducing heart failure in rats by injecting crude extracts of *Pavetta harborii* intraperitoneally (Hay, Pipedi, Schutte, Turner & Smith 2001). Administration of dried leaves or crude extracts to induce heart failure can, however, have limitations because of seasonal variations in plant toxicity resulting in variations in the degree of heart failure that develops. Theiler *et al. (*1923) found that factors such as soil type and seasonal climate changes caused variations in the toxicity of *Pachystigma pygmaeum* (= *Vangueria pygmaea*)

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and it should therefore be considered that this might also be the case for other species of this family.

Fourie, Erasmus, Schultz & Prozesky (1995) succeeded in isolating and describing the active compound which has become known as pavetamine. It became available in small quantities for further investigation.

Subsequent investigations have shown that pavetamine inhibits protein synthesis in the cardiac muscle of rats but that it has no effects on other tissues (Schultz, Fourie, Basson, Labuschagne & Prozesky 2001). The aim of this study was to investigate in rats whether pavetamine has cardiodynamic effects typical to those described previously for the dried material of *Pachystigma pygmaeum* and crude extracts of *Pavetta harborii* given to sheep and rats respectively.

METHODS

Experimental animals

The investigation conforms to the *Guide for the care and use of laboratory animals* (NIH publication No. 85-23, revised 1996). Ethics approval was obtained from the Animal Ethics Committee of the ARC-Onderstepoort Veterinary Institute.

Healthy, young, male Sprague Dawley rats ($n = 20$) of the same age were used and equally divided into a control and a treated group. They were kept individually in separate cages and had free access to water and nutritionally balanced rat cubes (Epol Pty. Ltd. SA). Pavetamine from dried *P. harborii* plant material was extracted by the method described by Fourie *et al.* (1995). The rats in the treated group each received an intraperitoneal injection of 4 mg/kg pavetamine in 0.5 mℓ saline on Day 1 and a second injection of 3 mg/kg in 0.5 mℓ saline on Day 20. The control rats only received similar quantities of normal saline administered by the same route. Cardiodynamic data were recorded for the individual experimental animals on 1 day from Days 35–39. These data were recorded for the individual rats over a period of 5 days as time constraints on the procedure limited the number of animals that could be done in 1 day.

The animals were anaesthetized by administering an intramuscular injection of 0.5 mℓ of a mixture of ketamine (100 mg/mℓ) (Kyron Laboratories Pty Ltd. Johannesburg, SA) and xylazine (2 %) (Premier Pharmaceuticals Co Ltd. Johannesburg, SA) at a ratio of 1:3. The anaesthesia was maintained throughout the procedure by further 0.1 mℓ injections at 20 min intervals or as needed. A constant anaesthetic plane was continually assessed by means of the tarsal pinch reflex.

The following surgical procedure and cardiodynamic monitoring on each rat were performed in a sterile environment on an operation table prewarmed to maintain the body temperature of the animals. Each rat was placed on its back and its right carotid artery surgically exposed and separated from the accompanying vagus nerve. Care was taken not to damage the nerve. A catheter (Cordis 2.5 F) was inserted into the left ventricle of the heart via the exposed artery. During this procedure care was taken to minimize blood loss. The catheter was connected to a fluid-filled Hewlett-Packard Quartz transducer which was connected to a Hewlett-Packard 8 channel recording system (HP7758) and the data obtained were stored on a magnetic tape for later analysis. The pressure recordings were monitored on an oscilloscope to confirm that the catheter was in the left ventricle.

The following indices were monitored and computed: peak left ventricular pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), heart rate (HR), the maximum rate of increase of left ventricular pressure ($+LVdP/dt_{max}$) indicating left ventricular (LV) contractility, the time constant T calculated from the LV pressure curve as the negative inverse of the slope of the regression line of the LV pressure versus LV -dP/dt with a variable pressure asymptote, which gives an indication of the rate of the left ventricular isovolumic relaxation (Weisfeldt, Weis, Frederiksen & Yin 1980), and the cardiac work (CW) that was calculated by multiplying HR and SBP. Directly after the last recordings the rats received a lethal dose (500 mg/kg) of sodium pentobarbitone (Kyron Laboratories (Pty) Ltd).

Statistical analysis was done with Gen Stat (2000) and the student t-test was used to compare the treated to the non-treated groups taking $P \leq 0.05$ as significant.

RESULTS

Fig. 1 shows that the rats receiving pavetamine had slower mass gains than those of control rats. After the second administration of pavetamine the treated rats showed no further mass gains up to the termination of the experiment.

Table 1 summarizes the effects of pavetamine on cardiac function. Both T and LVEDP were not signif-
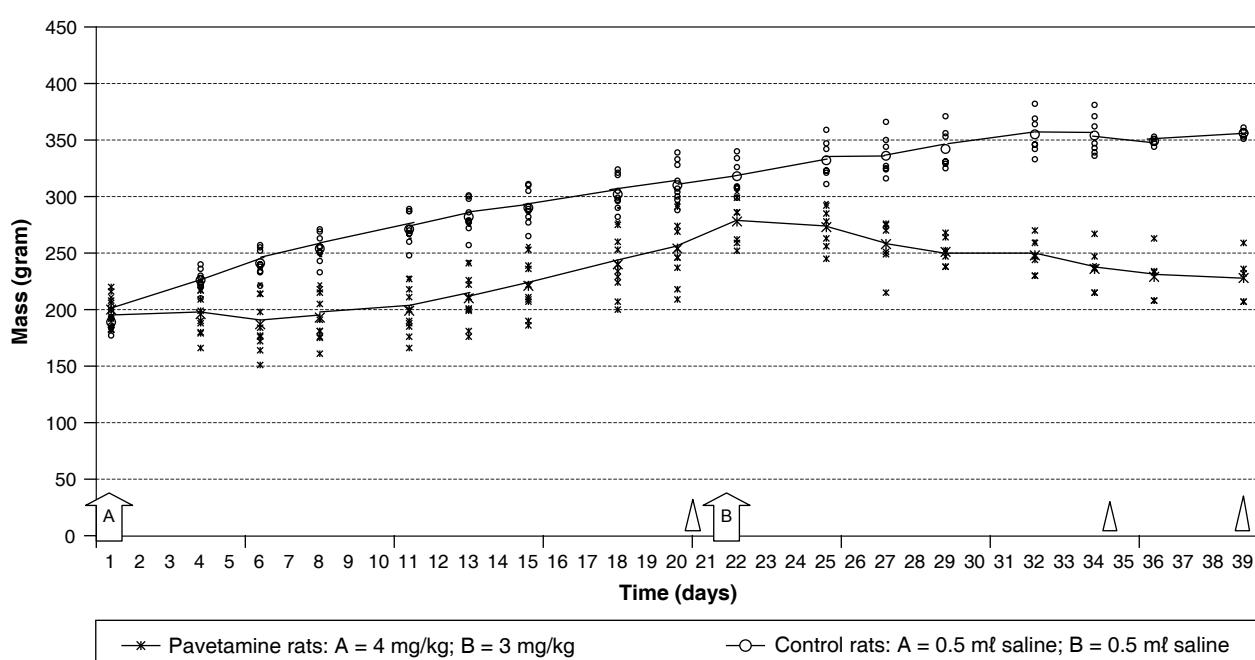
FIG. 1   The effects of pavetamine administration on rat body mass over a period of 39 days

TABLE 1 The cardiodynamic parameters of control rats and rats receiving pavetamine

	Control	Pavetamine	%Δ	*P*≤
T(ms)	52.11 ± 1.9	52.10 ± 1.6	0	NS
LVEDP (mmHg)	6.9 ± 1.3	6.4 ± 1.4	−7.2	NS
dP/dt$_{max}$ (mmHg.s^{-1})	2 746 ± 105	1897 ± 107	−30.9	0.001
CW (mm Hg.beats.min^{-1})	40 570 ± 1 799	28 010 ± 2 386	−30.9	0.001
LVSP (mmHg)	158.4 ± 7.8	119.5 ± 6.8	−24.5	0.002
HR (beats.min^{-1})	258 ± 11	231 ± 7	−10.5	0.05

See text for meanings of abbreviations

icantly affected by the pavetamine intervention. The other parameters that were measured were all significantly reduced. The reduction in dP/dt$_{max}$ and CW were identical at 30.9 % but since these two parameters are affected by different factors, this similarity is probably purely coincidental.

DISCUSSION

It is not clear how the pavetamine caused a decrease in the mass gain of the treated group. Previous studies did not reveal possible effects of these plant toxins on the mass of treated animals. In a study investigating the affect of pavetamine on protein synthesis in the different tissues of rats it was shown to have a detrimental effect on cardiac tissue but other tissues were not affected despite a loss in their mass (2 % within the first 24 h) (Schultz *et al.* 2001).

In another study where *Pavetta harborii* extracts were administered to rats, protein synthesis in car-

diac tissue was compromised to the extent that it lead to a degeneration in the myofilaments (Hay *et al.* 2001). A similar degeneration of myocardial fibres was also seen in sheep to which dried *Pachystigma pygmaeum* leaves had been administered (Schutte, Els, Booyens & Pienaar 1984). These pathological changes in the cardiac tissue could be the underlying cause of the reduced cardiodynamic function (systolic and diastolic) associated with the disease as described by Hay *et al.* (2001) and by Schutte & Du Plooy (1990). When a minimum critical dosage of the plant material is administered to animals and if enough time is allowed for the disease to progress fully, it will eventually reach a stage where typical clinical signs of congestive heart failure will manifest. These signs were described in detail by Pretorius & Terblanche (1967).

Our results show that the pavetamine significantly reduced the systolic component of the cardiodynamic function of the rats but did not affect the diastolic

component measured from T, nor did it influence the LVEDP. This is in contrast to the significant lengthening in T that was found in rats to which crude extracts of *Pavetta harborii* had been administered (Hay *et al.* 2001). A possible explanation for this could be that the concentration of the pavetamine used in this study was too low or that the time period over which the toxin was administered was too short, or both, to cause these additional changes associated with heart failure, namely a high LVEDP and a reduced diastolic function.

All the indicators of systolic function in our study were significantly reduced but the heart rate did not increase significantly, suggesting that the clinical signs usually associated with advanced congestive heart failure (Pretorius & Terblance 1967) had not yet fully developed.

Both CW and dP/dt_{max} were significantly decreased and the substantial reduction in LVSP implies that the reduction in dP/dt_{max} was sufficient to alter cardiac effectiveness. It is known that altered loading conditions of the heart can affect an index such as dP/dt_{max} (Broughton & Korner 1980). In our study only the afterload was significantly altered and this would therefore suggest that a slight overestimation of dP/dt_{max} would have occurred in the treated group. This fact combined with the lower LVSP would therefore suggest that this factor should not detract from our observations on dP/dt_{max}.

The HR was significantly reduced in the treated rats and this was unexpected in the light of a diminished contractile force. Similar observations were made in rats receiving crude extracts (Hay *et al.* 2001). However, in goats administered pavetamine resulted in tachycardia and ECG changes (Fourie *et al.* 1995). Likewise, in the sheep studies heart rate was initially not affected but later arrhythmias appeared and in the final stages of congestive heart failure tachycardia occurred (Pretorius & Terblanche 1967). Various changes in the ECG were described by Pretorius & Terblanche (1967). This would suggest that the toxin contained in the dried plant material affected the electrophysiology of the pacemaker cells. The authors of that paper suggested that ischaemia could be responsible for the observed ECG changes (Pretorius & Terblanche 1967). T-wave inversion was a common occurrence in sheep that received the dried plant material of *Pachystigma pygmaeum* (Pretorius & Terblanche 1967; Pretorius *et al.* 1973). T-wave inversion is also a well known phenomenon associated with hypokalemia (Ganong 2001) and since electrophysiological affects could also involve po-

tassium ions this aspect should be further investigated.

Therefore a possible effect of the toxin on the ions and the associated affect it might have on the membranes of the pacemaker cells should not be disregarded when the HR observations are considered. As mentioned before Pretorius *et al.* (1973) confirmed Ca^{2+} abnormalities in myocardial cells exposed to this toxin. Ca^{2+} abnormalities can technically also impact on the cell membranes of damaged myocardial cells via complex interactions in the Ca^{2+}/Na exchange system. This has been confirmed to take place in cases where myocardial cells were damaged in other ways than by this toxin (Pieske 1998).

In conclusion, the results suggest that pavetamine administered to rats diminishes their systolic function, but the effects on diastolic function and HR suggest that the rats were not in the advanced stages of congestive heart failure associated with a high LVEDP and compensatory tachycardia. Further dose response studies need to be done to determine the dose of pavetamine required to induce advanced congestive heart failure similar to the clinical picture described in sheep (Pretorius & Terblanche 1967; Kellerman *et al.* 2005).

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