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Cardiac evaluation after experimental intoxication by *Amorimia rigida* (Malpighiaceae) extracts in rabbits

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Abstract: Clinical and laboratory changes were evaluated in rabbits after intoxication by *Amorimia rigida*, a plant that causes sudden death. Nine New Zealand male rabbits, averaging 3.54 kg, were categorized into three groups (n = 3) and received, for eight consecutive days, the equivalent of 30 g/kg dry matter of *A. rigida* water-soluble (SG) and water-insoluble (IG) extracts via nasoesophageal route. The control group received water. There were no alterations in creatine kinase enzyme (CK), CK myocardial fraction (CKMB) or troponine I (cTnI). None of the animals had clinical or electrocardiographic (conventional and Holter) alterations. There were progressive decreases in the left ventricular ejection fraction and systolic fractional shortening. Doppler echocardiography alterations suggested a systolic dysfunction in the SG and IG groups and diastolic dysfunction in IG group. It was concluded that the soluble and insoluble extracts of *A. rigida* cause deficit of cardiac function.

Key words: toxic plant, *Amorimia rigida*, cardiac muscle profile, electrocardiography, echocardiography, rabbit.

INTRODUCTION

The genus *Amorimia* (Malpighiaceae) contains one of the 10 most important toxic plants in South America (1), best known of which is *Amorimia rigida* (*Mascagnia rigida*), well distributed in Minas Gerais state (Brazil), mainly in the northern and northeastern regions, where it is popularly known as "salsa-rosa" or "suma-roxa". It belongs to a group that causes acute intoxication that may provoke death preceded or not by a short period of clinical signs (2).

After *A. rigida* intake, animals may show apathy, anorexia, rigid walking, prolonged recumbence, muscle tremors and death, among other clinical signs. Significant alterations have not been found at necropsy of animals intoxicated by *A. rigida*. Multifocal infiltrate of lymphocytes in the myocardium associated with edema and congestion of myocytes was found in the histological exams (3).

Studies are necessary to elucidate which physiopathological mechanisms are involved in *A. rigida* intoxication that leads to sudden death. In spite of the existence of some reports of mortality in the absence of cardiac lesions, it is suggested that the main action of this plant in the heart may be due to its phytochemical characteristics that might affect cardiac function within a period insufficient to produce alterations detectable by optical microscopy (4).

To the best of our knowledge, no previous study has concurrently evaluated continuous electrocardiography (Holter), Doppler echocardiography and the cardiac muscle biochemical profile in animals after the intake of *A. rigida*. Thus, the present work aimed to ascertain the influence of this plant on the cardiac

system using the rabbit as the experimental model.

MATERIALS AND METHODS

Animal Assays

Nine six-month-old white New Zealand male rabbits weighing an average of 3.54 kg were used. Firstly, they were dewormed with 1% ivermectin (Mectimax, Agener União Saúde Animal, Brazil) and housed in 90x90x40 cm individual metal pens, following a quarantine period. They were given water and commercial food (Nature Multivita, Socil Evialsis) *ad libitum*. The experiment was approved by the Ethics Committee on Animal Experimentation (CETEA), Federal University of Minas Gerais, protocol number 187/08.

Preparation of *Amorimia rigida* Extracts

The 12 kg of *Amorimia rigida* mature leaves utilized was collected from the rows of toxic plants located at School of Veterinary Medicine, Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil. There is a voucher specimen of the plant deposited in the Herbário de Botânica based at the Institute of Biological Sciences at UFMG, maintained under the number 100819.

The extracts were crushed in a blender with ultra-pure water and sifted to obtain an aqueous solution, which was kept at 4ºC for 24 hours in order to form two distinct phases. They were then separated into two solutions: soluble and insoluble in water, both being concentrated in a rotary evaporator vacuum system at 70ºC, 80 rpm (Dia Pump Aspirated Compressor, CA model).

To calculate the dry matter (DM) equivalent and sample concentration, five *A. rigida* leaves were sampled, weighed and placed into the incubator for 30 minutes, in quintuplicate. After drying, they were weighed and the mean DM value of 35% was obtained. The final concentrations of the solutions were 4.42 g/mL of soluble extract and 2.71 g/mL of insoluble extract, pH 5.54.

The extracts were kept at -20ºC. They were maintained at 4ºC for 3h and homogenized in an ultrasound bath (Branson 1510, Bransonic Ultrasonic Cleaner, USA) for 10 minutes before being administered to the animals.

Experimental Groups

Rabbits were equally distributed into three groups: 30 mL of water – control group (CG); 26 mL of *A. rigida* extract, soluble in water, equivalent to 30 g/kg of plant DM – soluble group (SG); and 26 mL of *A. rigida* extract, insoluble in water, equivalent to 30 g/kg of plant DM – insoluble group (IG). All extracts were administered twice daily, via nasoesophageal probe (#6 urethral probe – Markmed): (8:30 am and 2:30 pm) for eight consecutive days.

Image Diagnosing Techniques (Cardiovascular Examinations) and Cardiac Muscle Biochemical Profile

Animals were clinically evaluated by electrocardiography (ECG) (electrocardiograph ECGPCVVET – Tecnologia Eletrônica Brasileira S.A.); continuous electrocardiography (Holter) (Cardiolight – Cardio´s); echocardiography (HP Sonos 100 CF echocardiograph – Hewlett Packard); as well as by the following serum biochemical exams: aspartate aminotransferase (AST) (Synermed); lactate-dehydrogenase (LDH); creatine kinase (CK) (Bioclin CK – NAC and CK-MB – Quibasa Química Básica, Brazil); MB CK fraction (CK-MB); and troponin I (cTnI) (Troponin I Test Bioeasy – Belo Horizonte, Brazil), before (T 0) and after the administration of the extracts (day 3: T1, day 5: T2, day 7: T3, and day 9: T4).

ECG was performed at T0, T1, T3, and echocardiography and Holter at T0 and T4. Only one animal of each group was submitted to Holter examination. Previously rabbits had been sedated with 1mg/kg midazolam maleate (Dormire, Cristália Produtos Químicos Farmacêuticos, Brazil) to minimize the stress and allow the examinations (5). Animals were shaved and electrodes for ECG were placed according to Tilley (6). Recordings were made in DI, at 50 mm/sec, and 2N (7). The QT interval was corrected using Carlsson formula (8). Electrodes for the Holter examination were placed on the thorax according to Oliveira *et al.* (9) being attached with bandages. Animals were using a cervical collar (10) and specific vest with a pocket where the Holter device was placed. Tracings were recorded throughout 14 hours. Echocardiography comprised bidimensional, M-mode, pulsed Doppler and color flow mapping evaluation methods as described by Stypmann *et al.* (11), considering the mean value of three measurements of each variable (12).

Statistical Analysis

The experiment was carried out in a random sample / sampling design, arranged in subdivided parcels, with the groups being used as parcels and times as sub-parcels. The results were submitted to analysis of variance (ANOVA) and means were compared by Duncan's test at 5% significance.

RESULTS AND DISCUSSION

None of the rabbits showed clinical signs of *Amorimia rigida* intoxication in agreement with Melo *et al.* (2) who also had not observed any behavioral alterations in mice after different intake levels of this plant. Conversely, Lago *et al.* (4) reported that after the administration of 20 g/kg of *A. rigida* (*Mascagnia rigida*) aqueous extract to 10 sheep, signs and symptoms of apathy, exercise intolerance, increased heart rate during rest, loss of appetite and pollakiuria were observed. Moreover, one animal died after five days.

The animals that received *A. rigida* extracts showed diminution ($p < 0.05$) of aspartate aminotransferase (AST) on the 3rd and 5th days after treatment, respectively, in the insoluble extract (IG) and soluble extract (SG) groups. The AST values for rabbits may vary from 14 to 113 U/L (13) and therefore all values obtained in the present study were within the threshold for that species (Table 1). There was a decrease ($p < 0.05$) of lactate-dehydrogenase (LDH) at T2, T3 and T4 (from 5th to 9th day) in SG animals when compared with T0. The IG rabbits presented a LDH decrease ($p < 0.05$) only on the 5th day (T2). LDH reference values for rabbits range from 34 to 129 U/L (14) and therefore the values measured in SG animals on the 5th and 7th days and in IG on the 5th day were below normality.

Guinea pigs intoxicated with *A. publiflora (Mascagnia publiflora)*, another *Amorimia* specie, presented higher AST values (119.6 U/L) when compared with healthy specimens (29.69 U/L) (15). However, Melo *et al.* (2) observed that *A. rigida* aqueous fractions (tannins, flavonoids, alkaloids and saponins) at 9 kg/g dosage did not cause AST alteration in mice, but higher dosages of 18 and 27 g/kg administrated for seven consecutive days caused significant increase of that enzyme. The same authors also evaluated

Table 1. Mean serum values of aspartate aminotransferase (AST – U/L) and lactate dehydrogenase (LDH – U/L) in rabbits submitted to gavage with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble *A. rigida* extract (IG) at different times

	Time (T)	Groups		
Variables*	(days)	Control	Soluble	Insoluble
	T zero	62.00 ± 26.46^{b}	88.00 ± 21.63^{a}	87.67 ± 16.62^{a}
AST	T1 – 3days	54.00 ± 34.83^{b}	77.00 ± 2.65^{ab}	44.00 ± 13.45^{c}
(U/L)	T2 – 5 days	98.00 ± 60.63^{a}	53.33 ± 14.84^{b}	59.67 ± 17.79^{abc}
	T3 – 7 days	78.00 ± 57.38^{ab}	60.67 ± 9.29^{ab}	45.67 ± 10.02^{bc}
CV(%): 43.69	T4 – 9 days	77.00 ± 43.86^{ab}	73.67 ± 30.35^{ab}	78.00 ± 33.45^{ab}
	T zero	57.00 ± 27.84^{a}	89.67 ± 24.83^{a}	63.67 ± 21.39^{a}
LDH	T1 – 3days	61.67 ± 22.81^{a}	55.00 ± 1.00^{ab}	38.70 ± 13.67^{ab}
(U/L)	T2 – 5 days	57.00 ± 6.00^{a}	29.63 ± 16.00^{b}	15.63 ± 2.49^{b}
	T3 – 7 days	43.33 ± 7.57^{a}	24.67 ± 2.52^{b}	52.67 ± 32.19^{a}
CV(%): 50.12	T4 – 9 days	67.67 ± 44.46^{a}	36.67 ± 3.21^{b}	48.33 ± 13.01^{ab}

CV: coefficient of variation
*Means followed by same letters do not statistically differ among the times (columns), submitted to the analysis of variance, SNK test (ALT) and Duncan test (LDH) ($p < 0.05$)

Table 2. Mean serum levels of creatine kinase* (CK – U/L), MB creatine kinase fraction* (CK-MB – U/L) and relative percent of CK-MB* (CK-MB/CK - %) in rabbits submitted to gavage with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble *A. rigida* extract (IG) at different times

	Time (T)	Groups		
Variable	(days)	Control	Soluble	Insoluble
CK (U/L) CV(%): 57.42	T zero	1112.07 ± 496.21	1006.73 ± 198.40	577.80 ± 190.73
	T1 – 3 days	928.03 ± 378.36	778.10 ± 250.56	669.40 ± 270.00
	T2 – 5 days	1776.80 ± 577.36	1195.73 ± 432.18	1021.57 ± 649.33
	T3 – 7 days	1927.03 ± 702.35	1559.73 ± 1111.17	1268.93 ± 1085.59
	T4 – 9 days	1905.87 ± 847.49	1165.43 ± 668.12	616.27 ± 205.03
CK-MB (U/L) CV(%): 64.77	T zero	363.73 ± 214.02	413.67 ± 178.01	230.43 ± 177.92
	T1 – 3 days	505.40 ± 346.66	276.30 ± 46.55	135.47 ± 55.25
	T2 – 5 days	361.03 ± 136.12	320.00 ± 165.85	213.17 ± 58.56
	T3 – 7 days	317.87 ± 88.62	224.50 ± 142.39	287.10 ± 212.00
	T4 – 9 days	486.27 ± 142.70	577.70 ± 578.02	300.30 ± 116.33
CK-MB/ CK (%) CV(%): 55,68	T zero	35.89 ± 27.49	43.68 ± 26.17	43.33 ± 29.88
	T1 – 3 days	51.19 ± 15.02	38.77 ± 15.38	25.66 ± 21.92
	T2 – 5 days	20.39 ± 5.59	25.40 ± 6.25	24.35 ± 9.47
	T3 – 7 days	17.20 ± 4.74	15.06 ± 4.33	27.44 ± 14.46
	T4 – 9 days	29.90 ± 15.18	45.01 ± 22.57	48.13 ± 2.95

CV: coefficient of variation

LDH and did not find significant alterations. AST is present in many tissues, including skeletal muscle and heart, and therefore is used as a marker of muscle lesion. However, as a mitochondrial and cytosolic enzyme, a more severe lesion is necessary to cause its release into circulating blood. Similarly, LDH is present in many tissues, including skeletal and striated muscles, mainly in rapid contraction muscles. Its low specificity leads to the evaluation of more specific cardiac markers such as creatine kinase (CK), MB creatine kinase fraction (CK-MB) and troponine, a subunit of muscle structure protein (14).

There were no differences (P>0.05) in CK, CK-MB and CK-MB/total CK ratio after *A. rigida* administration (Table 2). CK-MB/total CK ratio provides reliable information regarding myocardium injury, since an increase in CK-MB values followed by CK augmentation may be caused by physical exertion, for example. However, when an increase of CK-MB is not followed by an elevation of total CK, the percentage of this enzyme will be higher, thus confirming a specific lesion of cardiac muscle. Our results differ from those reported by Melo *et al.* (2), who found an increase of 70 to 84% in CK-MB after the administration of saponin and alkaloid fractions of *A. rigida* to mice, respectively.

Cardiac troponin I (cTnI) measurements using immunochromatographic test were also unable to detect myocardial lesion. Normal cTnI concentration in healthy rabbits ranges from 0.012 to 0.014 ng/dL (16). As the reagent concentrations in the immunochromatographic test exceeded 0.5 ng/mL, it is possible to infer the occurrence of myocardial injury with low-level cTnI release. However, the use of this marker to detect experimental cardiac lesions in rabbits revealed values of cTnI much higher than that threshold (17), with plasma concentrations of up to 2 ng/mL (18). Therefore, all data obtained here demonstrated that there was no damage of cardiac muscle fibers after the administration of aqueous extracts of *A. rigida*.

The mean heart rate (HR) of all nine animals at T0 was 210 (+/-15) bpm after midazolam administration. HR measured in New Zealand rabbits by telemetry (19) without medication presented a mean value of 218 (+/-4) bpm, which was similar to the value obtained with the sedative protocol proposed in the present study, indicating a close proximity to the resting HR physiological values.

A significant increase ($p < 0.05$) in the HR values was observed only in SG, from the 3rd to the 7th days, as displayed in Table 3. All HR means were within the expected range for the leporine species (7).

Similarly to what was observed in SG rabbits, sheep experimentally envenomed with *A. rigida* leaves in an aqueous suspension (20 g/kg) for three or seven days presented an increase of HR at rest and mainly during physical effort associated with a decrease of cardiac function (4). At least among humans and dogs (20), cardiac diseases are directly related to altered HR, which increases in intensity to compensate the insufficiency. In animals that presented sudden death associated with *A. rigida* intoxication, tachycardia was one of the signs cited immediately before the onset of death (2).

Cardiac pattern was sinusoidal in almost all animals and times, except in two IG specimens that presented sinusoidal arrhythmia, one at T1 (3rd day) and the other at T2 (5th day). Such arrhythmia does not have clinical relevance. ECG records did not present any alterations that could represent clinical importance in *A. rigida* intoxication showing normal P-QRS-T complexes.

The PR interval showed a statistically significant difference ($p < 0.05$) in groups and times of treatment (Table 4), when compared to the control group (CG). As to the groups, SG showed a significant PR decrease at all times when compared to CG. Data from IG were similar to CG only at T2 (5th day). With regard to time, PR decreased significantly in SG from the 3rd to the 7th days. This finding may indicate an elevation in the conductibility of the cardiac electric impulse (positive dromotropism), which was not reported in either CG or IG.

The PR interval in rabbits ranges from 60 to 90 ms (21). Hence, in the SG on the 3rd and 5th days, PR interval mean values were below the minimum threshold.

T-wave values differed little between the groups SG and IG ($P<0.05$) at T1 (Table 5). About 52.78% of ECG had T wave higher than 25% of the R wave, including T0. The T amplitude varied from 0.12 to 0.44, averaging 0.11mV at DI (21), from which it could be inferred that there was no clinical significance associated with alteration in T wave amplitude.

In our experiment, only one SG animal showed inversion of the T wave on the 3rd day (T2). It was also submitted to the Holter exam (previously described). The maximum ST-segment deviation never exceeded 1mm (or 0.1mV), as also observed by Levine and Bristol (21). The inversion of T wave polarity in serial ECG is not considered normal (22). These transitory T-wave alterations may indicate a myocardial disease and might occur in the absence of a clinically detectable heart disease. Other conditions associated with this alteration are myocardial hypoxia, hydroelectrolytic disturbances, metabolic diseases and intoxications (6).

Table 3. Heart rate (HR) (bpm) mean values in rabbits submitted to gavage procedure with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble *A. rigida* extract (IG) at different times

Time (T)	HR (bpm)*		
(days)	Control Group	Soluble Group	Insoluble Group
T zero	216.67 ± 7.57[a]	203.33 ± 27.32[b]	246.33 ± 38.21[a]
T1 – 3 days	236.00 ± 25.71[a]	257.67 ± 23.76[a]	231.33 ± 28.36[a]
T2 – 5 days	254.33 ± 22.55[a]	249.00 ± 13.75[a]	229.67 ± 22.68[a]
T3 – 7 days	231.33 ± 19.01[a]	244.67 ± 16.26[a]	231.33 ± 20.03[a]

Coefficient of variation = 10.42%
*Means followed by same letters do not statistically differ among the times (columns), according to analysis of variance and SNK test ($p < 0.05$)

Table 4. Mean durations of PR interval (msec) in rabbits submitted to gavage procedure with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble *A. rigida* extract (IG) at different times

Time (T)	PR (msec)*		
(days)	Control Group	Soluble Group	Insoluble Group
T zero	79.67 ± 7.02^{Aa}	69.00 ± 6.08^{Ba}	61.33 ± 2.52^{Ba}
T1 – 3 days	74.67 ± 5.69^{Aa}	54.33 ± 5.03^{Bb}	62.00 ± 2.65^{Ba}
T2 – 5 days	74.00 ± 4.58^{Aa}	55.67 ± 5.77^{Bb}	65.33 ± 4.51^{Aa}
T3 – 7 days	74.33 ± 5.51^{Aa}	60.67 ± 5.13^{Bb}	60.67 ± 3.51^{Ba}

Coefficient of variation (%) PR = 13.63

*Means followed by the same upper case letter n the lines and by the same lower case letter in the columns do not differ according to the analysis of variance and SNK test ($p > 0.05$)

Alterations in T wave amplitude were reported in intoxication by cardiac glycosides of *Tylecodon wallichii* in guinea pigs (23); however, these results occurred in association with alterations in the QRS complex and QT interval just before death, similar to the description of Saad *et al*. (15).

QRS durations did not differ ($p > 0.05$) in groups that received *A. rigida* extracts according to data shown in Table 6. The QRS complex varied between 43 and 59msec in the present study, within the possible range from 20 to 60 msec.

Although each animal was in an identical position when the ECGs were performed, changes were observed in P wave voltage, QRS complex and T waves including in the CG group. These alterations were also reported in ECG of 23 healthy rabbits, without chemical or physical contention, performed every 48h, for two weeks, at the same time and under the same conditions (21).

As to the QRS complex, there was individual variation in all animals, mainly in CG and particularly in SG and IG animals before the administration of *A. rigida* extracts. The same results were also demonstrated in sheep intoxicated by this plant (4). These alterations may be caused by the great variability of the vagal mechanisms and extreme mobility of the rabbit heart, which produces inevitable changes in placement (24) and, consequently, of the electrical axis of the heart or intrinsic electrochemical changes of the heart itself (21).

The QT interval is associated with the duration of the action potential of ventricular muscles and increases in cardiopathy (6, 20). However, the QT-interval duration is inversely proportional to that of HR and may generate inconsistencies in its analysis during tachycardia, since even if it is prolonged, this effect may be masked by HR increase, thus hampering the drawing of conclusions from its results. Also considering that

Table 5. T wave mean amplitude (mV) in rabbits submitted to gavage with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble *A. rigida* extract (IG) at different times

	Time (T)	Groups		
	(days)	Control	Soluble	Insoluble
T (mV)	T zero	0.16 ± 0.05^{A}	0.11 ± 0.09^{A}	0.06 ± 0.04^{A}
	T1 – 3 days	0.18 ± 0.06^{A}	0.06 ± 0.01^{B}	0.07 ± 0.05^{B}
	T2 – 5 days	0.13 ± 0.04^{A}	0.05 ± 0.07^{A}	0.05 ± 0.05^{A}
CV(%): 63.91	T3 – 7 days	0.13 ± 0.03^{A}	0.09 ± 0.03^{A}	0.07 ± 0.07^{A}

CV: coefficient of variation

Means followed by the same upper case letter in the lines and by the same lower case letter in the columns do not differ, according to the analysis of variance, logarithmic transformation +1 (R) and SNK test ($p > 0.05$)

Table 6. QRS complex mean duration in rabbits submitted to gavage with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble *A. rigida* extract (IG) at different times

Time (T)	QRS (msec)		
(days)	Control Group	Soluble Group	Insoluble Group
T zero	53.33 ± 4.93[ab]	56.33 ± 3.79[a]	48.67 ± 5.13[a]
T1 – 3 days	52.67 ± 2.89[ab]	54.00 ± 1.73[a]	45.33 ± 1.53[a]
T2 – 5 days	48.00 ± 3.00[b]	53.33 ± 1.53[a]	47.00 ± 2.65[a]
T3 – 7 days	56.00 ± 1.73[a]	51.00 ± 5.29[a]	48.00 ± 4.00[a]

Coefficient of variation (%) QRS = 8.93
Means followed by the same lower case letter in the columns do not differ according to the analysis of variance and SNK test ($p > 0.05$)

HR differs in rabbits, it is important to exclude the effect of HR on the QT interval.

The QT reference values for adult New Zealand rabbits, corrected by the Carlsson formula, range from 142 to 157msec (25). As the QT (U) extrapolation method was used in the present study, but not by Wang *et al.* (25), there is a trend toward an increase of the interval. When comparing the conventional and the extrapolation methods, several authors (26) also observed the increase of this interval in QT (U) extrapolation.

In comparison to GC, the results indicate that *A. rigida* did not cause cardiac alteration sufficient to increase QTc (Table 7). However, it should be emphasized that a low degree of the intoxication may not have been sufficient to cause the occurrence of increase QTc. Thus, it may be supposed that *A. rigida* did not cause abnormalities in the ventricular action potential and if they occurred they were very slight and could not be demonstrated, probably due to HR increase (4).

All Holter exams, either at T0 or after intoxication (9th day), did not show rhythmic disturbances or alterations of conduction that may characterize arrhythmia in these rabbits.

The maximum speed of the aortic flow was lower during the times ($P<0.05$) on the 9th day in IG animals (Table 8). A decrease was also observed in the SG group, even though it was not significant. There was no difference of the maximum speed in mitral flow in relation to times or groups ($p>0.05$), although the mean was greater in IG. The high HR did not allow the individualization of the E or A mitral waves.

There was no difference ($p < 0.05$) among the groups and the times regarding %ΔD and ejection fraction (EF) of the left ventricle (Table 9). However, the EF and the %ΔD showed a decrease of 4 and 7% between T0 and T9, respectively, in CG animals, while in the treated animals (SG and IG), the respective decreases of EF and %ΔD were 15.4% and 23.1% in SG and 14 and 20.5% in IG. The reduction in the ejection fraction and shortening indicates that

Table 7. Mean values of QTc interval (msec) in rabbits submitted to gavage with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble *A. rigida* extract (IG) at different times

	Time (T)	Groups		
	(days)	Control	Soluble	Insoluble
QTc (msec)	T zero	171.30 ± 13.98	166.78 ± 4.23	163.83 ± 10.21
	T1 – 3 days	172.92 ± 5.58	160.49 ± 4.77	163.20 ± 4.98
	T2 – 5 days	168.30 ± 15.47	165.93 ± 7.23	166.42 ± 6.92
CV(%): 5.10	T3 – 7 days	165.88 ± 10.34	166.78 ± 11.07	165.16 ± 10.43

CV: coefficient of variation

Table 8. Mean maximum flow velocities in aortic valve (VmaxVA - cm/s) and mitral valve* (VmaxVM - cm/s) in rabbits submitted to gavage procedure with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble *A. rigida* extract (IG) at different times

	Time (T)	Groups		
	(days)	Control	Soluble	Insoluble
VmaxVA (cm/s) CV(%): 17.39	T zero	91.99 ± 4.97a	99.70 ± 4.11a	90.42 ± 5.28a
	T1 – 9 days	89.50 ± 2.68a	87.88 ± 4.30a	74.46 ± 14.29b
VmaxVM (cm/s) CV(%): 10.94	T zero	60.75 ± 8.59	67.45 ± 5.54	77.75 ± 13.68
	T1 – 9 days	68.12 ± 6.24	66.51 ± 15.44	85.19 ± 11.65

CV: coefficient of variation

the ventricular systolic function was affected (12, 27).

The literature shows a great variation in the reference values for EF and the other echocardiographic variables. Stypmann *et al.* (11) used the formula of Fontes-Souza *et al.* (28), the cubic formula to obtain EF (which is another factor), besides another anesthetic protocol, the conjunction of which prevents the comparison of the values obtained. In another experiment by our research group (5), EF and %ΔD were respectively 75.05±5.76% and 40.68±5.08% in rabbits sedated with midazolam (1 mg/kg IM). According to this reference and the CG values, there was a decrease of EF and %ΔD after treatment in SG and IG animals.

A decrease in EF and %ΔD has already been observed in animals intoxicated by *A. rigida* (*Mascagnia rigida*) (4). These authors found %ΔD values of 32.4% and 29.5% in sheep intoxicated for three and seven days, respectively. The control group values did not change. In our experiment, one SG rabbit presented a 39% reduction in %ΔD (from 43.7% to 26.5%) versus 34% in one IG rabbit (from 43.2% to 28.6%).

The final diastolic and systolic volumes of the left ventricle differed significantly ($p < 0.05$); however, the systolic volume was increased in SG and IG animals, whereas CG showed a decrease (Table 10).

The left atrium:aorta ratio (LA/AO) showed a difference ($P<0.05$) among the times in IG animals (Table 11), indicating enlargement of LA. The GI rabbits presented the lowest value of this ratio at T0, revealing a difference after the treatment.

LA length is greatly influenced by the same factors that determine the ventricular distention (pre-load, post-load, contractility, distensibility, contraction and HR) and thus constitutes a stable

Table 9. Mean of the systolic shortening percent* (%ΔD - %) and ejection fraction * (EF - %) of the left ventricle of rabbits submitted to gavage with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble A. rigida extract (IG) at different times

	Time (T) (days)	Groups		
		Control	Soluble	Insoluble
EF (%) CV(%): 9.75	T zero	70.39 ± 3.95	73.99 ± 7.37	77.37 ± 0.93
	T1 – 9 days	73.78 ± 2.41	62.57 ± 8.07	66.57 ± 6.40
%ΔD (%) CV(%): 14.46	T zero	36.66 ± 2.96	40.09 ± 6.23	42.13 ± 1.36
	T1 – 9 days	39.22 ± 2.11	30.83 ± 5.48	33.47 ± 4.80

CV: coefficient of variation

Table 10. Mean of the final diastolic volume* (LVFDV - mL) and final systolic volume* (LVFSV - mL) of left ventricle in rabbits submitted to gavage with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble *A. rigida* extract (IG) at different times

	Time (T)	Groups		
	(days)	Control	Soluble	Insoluble
LVFDV (mL) CV (%): 32.94	T zero	5.07 ± 0.96	5.62 ± 1.21	4.06 ± 1.98
	T1 – 9 days	4.02 ± 0.50	4.54 ± 2.44	3.39 ± 0.96
LVFSV (mL) CV (%): 42.24	T zero	1.48 ± 0.31	1.42 ± 0.37	0.91 ± 0.42
	T1 – 9 days	1.05 ± 0.14	1.77 ± 1.11	1.11 ± 0.26

*There was no statistical difference according to the analysis of variance ($p > 0.05$)

parameter that reflects the duration and severity of the diastolic function (29, 30). It is also a good predictor of adverse events in several clinic situations, such as diastolic dysfunction (31,32), independently of the presence of cardiovascular disease, left ventricle systolic dysfunction and ventricular hypertrophy (32). Alteration of LA/AO ratio provoked by LA enlargement in IG rabbits indicates cardiac diastolic damage of ventricular complacency or relaxing, which may alter the EF (33). Due to the resistance against ventricular distention, the augmented LA contracts more (Frank-Starling law), provoking increase of the atrial flow velocity as previously observed. However, the fact that the entire volume does not fit in the left ventricle causes atrial distention in order to receive the venous return (pre-load overload).

Despite the possibility that *A. rigida* administration may provoke cardiac damage, the results of the echocardiography indicate that the plant affects cardiac function. The post-intoxication decreases of ejection and shortening fractions and aortic flow velocity observed in both SG and IG rabbits reflect a systolic dysfunction of the left ventricle.

The diminished contractility of the left ventricle causes decrease in the ejection volume accounting for the increase in its final systolic volume, thus reducing the cardiac debt (27). In IG animals, it was not followed by an increase of the diastolic volume due to diastolic dysfunction, which may have altered the ventricular distention. In SG animals, the measuring of the final diastolic and systolic volumes after the treatment were impaired by the high standard deviation, although a high elevation in the final systolic volume was recorded. This finding revealed a slight increase compared to the CG group and corroborates the decrease in %ΔD.

CONCLUSIONS

The administration of 30 g/kg of *Amorimia rigida* aqueous extract did not cause any alteration in heart muscle biochemical profile or in the electrocardiographic exams (conventional

Table 11. Left atrium:aorta ratio (LA/AO) in rabbits submitted to gavage with ultra-pure water (CG), water-soluble *A. rigida* extract (SG) and water-insoluble *A. rigida* extract (IG) at different times

	Time (T)	Groups		
	(days)	Control	Soluble	Insoluble
LE/AO CV(%): 13.34	T zero	1.44 ± 0.11[a]	1.79 ± 0.03[a]	1.26 ± 0.19[b]
	T1 – 9 days	1.64 ± 0.22[a]	1.63 ± 0.09[a]	1.63 ± 0.08[a]

Means followed by the same lower case letter in columns do not differ according to the analysis of variance and SNK test ($p > 0.05$)

and Holter) of the experimental rabbits.

Either a decrease in the relaxing or an augmentation of the ventricular complacency provokes a diastolic deficit in the rabbits that received water-insoluble *A. rigida* extract; and a systolic deficit in animals of both groups, detected by Doppler echocardiography.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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ETHICS COMMITTEE APPROVAL

The present study was approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais (UFMG) (CETEA) under the protocol n187/2008, and followed the protocols of the International Society of Toxinology and the Brazilian Society of Science in Laboratory Animals.

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