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BIOMEDICAL SCIENCES

Effect of essential oil of *Alpinia zerumbet* on cardiovascular and autonomic function in rats with isoproterenol induced acute myocardial infarction

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Abstract: *Alpinia zerumbet* is a plant popularly used to treat hypertension and anxiety. Studies with *Alpinia zerumbet* demonstrate antihypertensive and vasodilator effects, among others. The objective of this study was to analyze the effect of essential oil of *Alpinia zerumbet* (EOAz) on cardiovascular and autonomic function in rats with isoproterenol-induced myocardial infarction. Male Wistar rats (n=32) were equally allocated into four groups: Control, ISO (150mg/kg, subcutaneous), EOAz (100mg/kg by gavage), ISO+EOAz. The rats were evaluated for cardiovascular and, autonomic parameters, electrocardiogram, and infarct size. EOAz was not able to reduce the electrocardiographic variations induced by ISO. Heart rate variability showed a decrease in sympathetic modulation on the heart in the groups treated with EOAz. The cardiopulmonary reflex induced by serotonin invoked a superior blood pressure variation at the 2 µg/kg dose in the EOAz treated groups, while the heart rate variation was significantly higher at the 16 µg/kg dose, when compared to other doses, in all groups, except EOAz+ISO. The sympathetic vagal index was higher in ISO group than in control. EOAz did not reduce the infarct size. We conclude that pretreatment with EOAz does not reverse the hemodynamic and electrocardiographic damage caused by isoproterenol but does reduce sympathetic modulation.

Key words: Medicinal plants, myocardial infarction, sympathetic nervous system, cardiovascular system.

INTRODUCTION

Alpinia zerumbet (Pers.) Burtt & Smith is a plant belonging to the family Zingiberaceae, order Zingiberales, native to tropical regions, especially southern and southeastern Asia. The essential oil of *Alpinia zerumbet* (EOAz) contains a large amount of mono and sesquiterpenes, with a high concentration of cineole and terpineol (Cunha et al. 2013).

Previous studies have demonstrated several pharmacological effects of *Alpinia zerumbet*, including hypotensive (Lahlou et al. 2002), vasodilator (Cunha et al. 2013), and negative inotropic and chronotropic effects (Santos et al. 2011). The mechanism of action of *Alpinia zerumbet* is mainly via the blockade of calcium channels (Cunha et al. 2013).

Lobo-Filho et al. (2011) evaluated the effect of EOAz on rats with isopreterenol induced infarction and found that pre-treatment with

EOAz attenuated the elevations of Glutamate Oxaloacetate Trasnsaminase (GOT) and troponin I, attenuated the elevated number of neutrophils, and preserved the levels of catalase and glutathione in the myocardium. However, EOAz had no effect on mortality, animal weight variation, serum Glutamic Pyruvic Transaminase (GPT) levels, serum hemoglobin levels, leukocyte counts, or serum levels of renal function markers.

A large number of toxicological studies (including human) have demonstrated an absence of toxicity from *Alpinia zerumbet* essential oil, leaf extract and tea (Cavalcanti et al. 2012).

The development of phytotherapeutic formulations directed to a diversity of pathological conditions, including cardiovascular diseases is of fundamental importance, as long as their efficacy is proven by scientific studies. Thus, the objective of this study was to verify the effect of EOAz pretreatment on the cardiovascular, electrocardiographic and autonomic parameters of rats with isoproterenol induced acute myocardial infarction.

MATERIALS AND METHODS

Experimental Animals

This study was approved by UFC animal research ethics committee (*Comissão de Ética em Pesquisa Animal* – CEPA); approval number 56/15. The study was conducted in accordance with the recommendations of the Brazilian College of Animal Experimentation (COBEA) and in accordance with Law N°. 11794 of the Brazilian Guideline for Care and Use of Animals for Scientific and Educational purposes (DBCA). Male, healthy Wistar rats from the animal research unit at the Federal University of Ceará (UFC) were used. Animals weighing 250-300 grams were selected and were maintained in a light-dark cycle of 12-12 hours with free access to food and water.

Essential oil of *Alpinia zerumbet* (EOAz)

The essential oil of *Alpinia zerumbet* was extracted from leaves collected on the Vila Nova site, located in the district of Ladeira Grande, in the municipality of Maranguape, Ceará. A sample of the plant was identified in the Prisco Bezerra Herbarium, of the School of Agronomy, UFC, being deposited under voucher number 50312.

The EOAz was obtained using the steam distillation technique, which is commonly used to obtain oils from leaves. This type of distillation is used to isolate substances that decompose in the vicinity of their boiling point and that are insoluble in water or in their steam distillation (Cunha et al. 2013).

EOAz was extracted by hydrodistillation in Clevenger-type glass apparatus. The essential oil was analyzed by gas chromatography coupled with mass spectrometry (GC-MS) and flame ionization detection (GC-FID) according to method described (Cavalcanti et al. 2012). GC-MS analysis was carried out on a Shimadzu QP5050 instrument equipped with non-polar OV-5 fused silica capillary column, while GC-FID analysis was carried out on a Shimadzu GC 2010 Plus instrument provided with non-polar CP-Sil-8 fused silica capillary column (Cunha et al. 2013).

GC-MS and GC-FID analysis of EOAz obtained by chromatographic separation permitted identification of thirty-two constituents, including 25 monoterpenes, 6 sesquiterpenes and one alkane. The essential oil was composed mainly 1,8-cineol (22.40%), *p*-cymene (18.91%) and terpinen-4- ol (17.32%). Some minor compounds such as camphene, phelandrene, *p*-menth-2-en- 1-ol and γ-cadinene could be detected only after chromatographic fractionation (Table I) (Cunha et al. 2013).

Table I. Chemical composition, kovats retention indices and relative area of the constituents of EOAz.

Volatile components	IK[a]	Relative area (%) [b]
α-Thujene	931	3.84
α-Pinene	940	2.12
Sabinene	978	9.90
β-Pinene	983	3.60
Myrcene	992	0.80
α-Terpinene	1021	2.50
p-Cymene	1029	18.91
Limonene	1034	2.42
1,8-Cineol	1037	22.40
γ-Terpinene	1063	11.42
Terpinolene	1092	1.18
Linalool	1101	1.04
Terpinen-4-ol	1182	17.32
α-Terpineol	1193	0.78
β-Caryophyllene	1424	1.11
Caryophyllene oxide	1587	0.66

[a] IK-Kovats retention indices calculated from a homologous series of n-alkanes (C_7-C_{30}) analyzed on a CP-Sil-8 column. [b] Relative area percentage determined by GC-FID.

Myocardial Infarction Model

Isoproterenol was used to induce myocardial infarction (MI) at a dose of 150mg / kg, injected subcutaneously in the right posterior quadrant of the animal's back on two consecutive days, with a 24h interval between administrations (Lobo-Filho et al. 2011).

The trial consisted of 32 animals randomly distributed in 4 experimental groups of 8 rats each: (I) Control - water by gavage and physiological saline solution 0.9% (SS); (II) ISO - water by gavage and isoproterenol; (III) EOAz - EOAz by gavage and SS; (IV) ISO + EOAz - EOAz by gavage and isoproterenol. Gavage administrations were 0.5 ml of water or EOAz at 100 mg / kg, diluted in water up to 0.5 ml, and were administered for 7 days. Isoproterenol or SS were administered via subcutaneous injection in a volume of 0.2 ml during days 6 (D6) and 7 (D7) of the treatment.

The dose of EOAz used in this study was based on previous studies (Cunha et al. 2013).

Experimental Protocol

Evaluation of cardiovascular parameters

The rats were anesthetized with xylazine hydrochloride (10 mg / kg) and ketamine hydrochloride (90mg / kg) intraperitoneally. After anesthesia, the procedure for insertion of the properly calibrated Mikro-Tip pressure catheter (Model SPR-671, Millar Instruments, Houston TX) into the left femoral artery was performed. For the administration of drugs, cannulation (PE 10) of the left femoral vein was performed. The electrodes were then placed to capture the electrocardiographic signal using DII derivation.

A stabilization period of 10 minutes prior to initial data collection was observed. Fifteen minutes of baseline data were then acquired prior to initiating the autonomic system evaluation protocol (Gomes et al. 2017, Sanches et al. 2009).

For the measurement of the mean blood pressure (MBP), and diastolic (DBP) and systolic (SBP) blood pressure, a Mikro-Tip pressure catheter was used. The Bio Amp acquisition system (AdInstruments, Australia) was used to perform the electrocardiogram (ECG), which recorded the ECG by the DII lead, using subcutaneous needles electrodes attached to alligator clips. The ECG and arterial pressures were processed and analyzed using the PowerLab system for LabChart v8.0.8 software (AdInstruments, Australia). Heart rate (HR) was

calculated from ECG and BP using LabChart v8.0.8 software (AdInstruments, Autralia).

To evaluate the myocardial workload, the rate-pressure product (RPP) was calculated by multiplying SBP by HR (Fornitano & Godoy 2006).

Evaluation of autonomic function

In the baroreflex evaluation, increasing and alternate doses of phenylephrine (0.5; 1 and 2ug / kg) and sodium nitroprusside (5, 10 and 20ug / kg) were used (Gomes et al. 2017, Sanches et al. 2009).

Afterwards, cardiopulmonary receptors were evaluated through the Bezold-Jarisch reflex with serotonin infusion (2, 4 and 16ug / kg) at intervals of up to five minutes between doses (Sanches et al. 2009).

Finally, function of the sympathetic and parasympathetic autonomic nervous system was evaluated through the sympathetic-vagal index (SVI) with a dose of atropine (3mg / kg) followed by a dose of propranolol (4mg / kg) to obtain the heart rate in order to calculate the intrinsic heart rate (IHR) (Gomes et al. 2017, Sanches et al. 2009, Mostarda et al. 2014). Pharmacological blockade with atropine and propranolol can be used in both animal and clinical trials to quantify the influence of autonomic system on heart rate and cardiac output. The double pharmacological block allows the determination of the IHR and the SVI, which is calculated by the ratio of baseline HR and IHR. An SVI> 1 means a predominance of sympathetic tone over the parasympathetic, and an SVI <1 reflects a preponderant vagal tone over the sympathetic one (Gomes et al. 2017, Sanches et al. 2009).

In the present study, it was observed that, there was no significant difference between the three autonomic protocols (baroreflex, cardiopulmonary receptors and autonomic nervous system).

Heart rate variability was obtained from the SBP and HR recorded in Labchart v8.0.8, using 10 minutes of recording after 15 minutes of stabilization to calculate the variabilities. Time in seconds, SBP and HR data were entered in to Microsoft Excel (2013), where a treatment was performed to exclude discrepant values, which are values greater or less than the mean plus or minus, respectively, two standard deviations.

After exclusion of the discrepant values, HR values were used to calculate pulse intervals (PI), using the following formula PI = (60/HR)*100 (Fornitano & Godoy 2006).

SBP and PI data, and time were analyzed using the CardioSeries software v2.4 (PENTEADO, D. Medical College from São Paulo), which calculates heart rate variability (HRV) and blood pressure variability (BPV) in the time domain, providing the parameters mean, standard deviation and variance for BPV, and mean, standard deviation, variance and RMSSD (Root-Mean of square successive NN interval difference) for HRV (Fazan et al. 2008).

In the frequency domain the wave components were divided into very low frequency (VLF) (0.02 to 0.2Hz), low frequency (LF) (0.2 to 0.8Hz) and high frequency (HF) (0.8 to 3 Hz) (Fazan et al. 2008). The parameters obtained in the frequency domain were the percentage values of the bands VLF, LF and HF for BPV and HRV, as well as the LF / HF ratio for HRV.

Evaluation of infarct extent

The heart was removed shortly after collecting the autonomic system data, and was washed twice in 0.9% SS at 37 °C to remove the blood from the cardiac chambers. The organ was then frozen for 30 minutes to allow a more precise cut, which was performed transversely with a scalpel. The two-millimeter-thick slices were then placed in 3ml of Triphenyltetrazolium Chloride (TTC) at a concentration of 1% and

preheated at 37 °C for 20 minutes. After staining, the sections were removed from the solution and placed in 10 ml of 10% buffered formalin to increase the contrast of the infarcted area (Chrastina et al. 2014).

In order to photograph the sections, a microscope (model MC-M2222, DF Vasconcellos SA, São Paulo) with a 10-fold magnification, coupled to a digital camera was used. The sections were placed with the apical face down on a black background and submerged in water to avoid light reflections. The images of the sections were standardized by consistently positioning the right ventricle part on the right of the slide (Chrastina et al. 2014).

The images were stored in BMP format with a size of 1024x768 pixels. The total area of the slice and the infarcted area were then marked and analyzed from the images using ImageJ® software. For each slice, the weight of the infarcted region was obtained by dividing the infarcted area by the total area, multiplied by the slice weight. The extent of infarction was then calculated from the ratio between the sum of the infarcted area weights of all the slices and total heart weight, expressed as a percentage.

Drugs and reagents

All drugs and reagents used in this study (phenylephrine, sodium nitroprusside, serotonin, propranolol, atropine, isoproterenol, triphenyltetrazolium chloride) were supplied by Sigma Aldrich, St. Louis, MO, USA. The anesthetics used in the research (ketamine and xylazine) were produced by Syntec do Brasil (São Paulo, Brazil).

Statistical analysis

Continuous and discrete variables were first analyzed by the Kolmogorov–Smirnov test to verify the normality of distribution. As such criteria were met in all analyses, the mean and standard deviation (SD) were calculated for descriptive statistics, and parametric tests were applied for analytical statistics. Thus, comparisons of the cardiovascular and autonomic parameters between the four groups (Control, ISO, EOAz and EOAz+ISO), were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test to compare all pairs of groups. In the analysis of HR and MBP variation induced by serotonin stimulated cardiopulmonary reflex, comparisons between different serotonin doses within the same group were carried out using repeated measures ANOVA followed by Tukey's multiple comparison test. An unpaired *t* test was used to compare infarction size between the ISO and EOAz+ISO groups. In all analyses, two-tail tests were employed and the significance level was set at 0.05. All statistical procedures were performed and graphs plotted using GraphPad Prism® version 5.00 software for Windows® (GraphPad Software, San Diego, California, USA, 2007).

RESULTS

Cardiovascular parameters

SBP, DBP, and MBP were not significantly different between groups (Table II). HR and RPP (Table II), showed a significant difference only between the EOAz and ISO groups, with both HR ($P < 0.01$) and RPP ($P < 0.05$) being higher in the EOAz group.

Eletrocardiographic parameters

The duration of the QRS complex was increased in the ISO and EOAz + ISO groups when compared to the control group (Figure 1a). The QTc interval was increased in the ISO and EOAz + ISO groups when compared to the other two groups (Figure 1b). R wave amplitude was significantly lower in the ISO, EOAz and EOAz + ISO groups when

Table II. Cardiovascular parameters: Systolic (SBP), diastolic (DBP) and mean blood pressure (MBP), heart rate (HR) and rate-pressure product (RPP).

Parameter	Control Mean ± SD	ISO Mean ± SD	EOAz Mean ± DP	EOAz+ISO Mean ± SD	Significance (ANOVA)
SBP (mmHg)	118.51 ± 17.39	100.65 ± 7.36	114.58 ± 29.52	91.33 ± 19.20	**P = 0.0399***
DBP (mmHg)	78.75 ± 12.49	73.12 ± 6.36	73.05 ± 28.94	62.98 ± 13.61	P = 0.3532
MBP (mmHg)	96.49 ± 14.42	84.33 ± 6.89	92.12 ± 29.02	74.50 ± 16.51	P = 0.1130
HR (bpm)	299.84 ± 70.22	245.42 ± 59.04	351.07 ± 29.29[a]	322.10 ± 68.19	**P = 0.0098**
RPP ($mmHg.bpm.10^{-3}$)	35.72 ± 11.89	25.20 ± 5.89	39.93 ± 11.63[b]	30.32 ± 7.88	**P = 0.0278**

Data correspond to measurements obtained from 8 animals in each group. [a](P < 0.01) and [b](P < 0.05) compared to ISO group (Tukey's test). *Statistically significant differences between the four groups by ANOVA (P=0.0399), but these differences weren't confirmed by Tukey's test (P>0.05).

compared to the control group (Figure 1c). The ST segment, when compared to the Control and EOAz groups, was increased in the ISO and EOAz + ISO groups (Figure 1d).

Heart Rate Variability

Analysis of the time domain parameters of HRV showed a significant difference in the mean parameter in the EOAz (P <0.01) and EOAz + ISO (P <0.05) groups, with both being significantly lower than the observed mean in the ISO group. No significant differences were observed in the standard deviation, variance or RMSSD, as can be seen in Table III.

Analysis of the frequency domain parameters of HRV showed significantly lower values in the LF component, both the EOAz and EOAz + ISO groups, had a significantly lower percentage than the ISO group (P <0.05) (Table III).

Sistolic Blood Pressure Variability

A statistically significant difference in BPV was not observed in the time domain parameters (Table IV). For the frequency domain parameters, a statistically significant difference was observed in the percentage of LF (P = 0.0282) and HF (P = 0.0407) bands (Table IV). A statistically significant difference was observed only in the EOAz group, which had a significantly lower LF percentage, and a significantly higher HF percentage than the ISO group.

Autonomic parameters

Regarding bradycardia related to baroreflex sensitivity with phenylephrine administration, no statistically significant differences were found between groups (ANOVA: F = 0.6808; P = 0.5712). Concerning tachycardia related to baroreflex sensitivity with nitroprusside administration, no statistically significant differences were found between groups (Table V).

A significant statistical difference in blood pressure was observed in the second and third dose of serotonin (4 and 16 µg / kg) when compared to the first dose within the same group for two groups, group EOAz (P <0.05), and group EOAz + ISO (P <0.001). Regarding the HR variation in the cardiopulmonary reflex stimulated by serotonin in sequential and increasing doses (Bezold-Jarisch). The letters a (P <0.05) and b (P <0.05) denote statistically significant differences

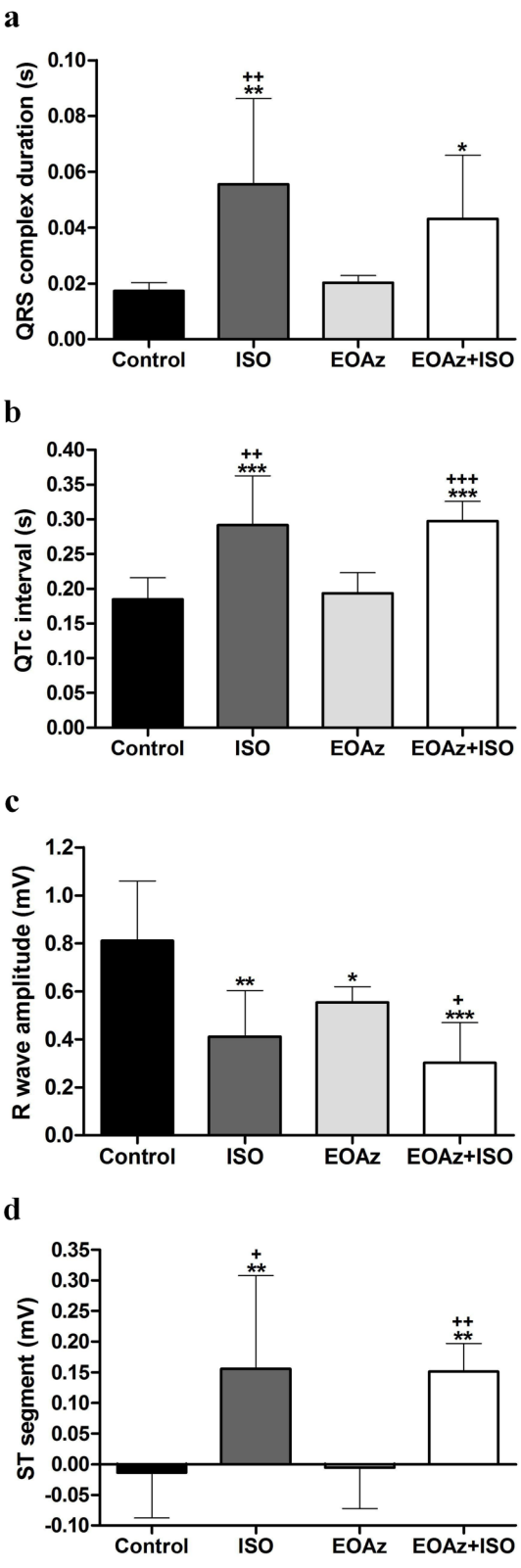

Figure 1. Electrocardiographic parameters in Control, ISO, EOAz and EOAz+ISO groups evaluated by intradermic needles connected in ECG electrodes in DII derivation. The measured parameters were the QRS complex duration in seconds (a), QTc interval duration in seconds (b), R wave amplitude in millivolts (c), the ST segment in millivolts (d), and representative ECG traces of each group (e). Data expressed as mean and SD from measurements realized in 8 animals from Control, EOAz and EOAz+ISO groups, and in 6 animals from ISO group. *P<0.05, **P<0.01, ***P<0.001 compared to Control group; +P<0.05, ++P<0.01, +++P<0.001 compared to EOAz group.

in relation to the Control and EOAz groups, respectively (Table VI).

The analysis results of the IHR and SVI data are shown in Figure 2a and 2b, respectively. It was verified that IHR in the ISO group was significantly lower than that measured in the Control (P <0.01) and EOAz (P <0.01) groups. The sympathetic-vagal index verified in the ISO group was significantly higher than that measured in the Control group (P <0.01).

Figure 3 shows photographs of the TTC-stained rat heart sections from the four treatment groups. The areas of infarction (pale) are shown in the ISO and EOAz + ISO groups, however no pale area was visible in the Control or EOAz groups, evidencing an absence of ischemic areas. The pale areas were more localized in the ISO group than in the EOAz + ISO group where these areas were more diffuse. No statistically significant difference was observed in the extent of MI, Figure 4 between the ISO (18.58 ± 7.17%) and EOAz + ISO (16.45 ± 6.15%) groups (P = 0.5344).

DISCUSSION

This research aimed to verify the effect of a phytotherapeutic substance, *Alpinia zerumbet* essential oil, in its totality, as a potential future treatment for patients, and as such, the study did not include pre-treatment with any isolated substance. The search for natural agents that

Table III. Heart rate variability parameters at the time and frequency domain.

Parameter*	Control	ISO	EOAz	EOAz+ISO	Significance (ANOVA)
Time domain (Mean ± SD)					
Mean (ms)	209.21 ± 55.28	249.31 ± 51.14	172.75 ± 13.10[a]	183.25 ± 29.18[b]	**P = 0.0040**
Standard deviation (ms)	4.94 ± 3.32	4.50 ± 3.50	4.89 ± 5.33	4.16 ± 2.85	P = 0.9741
Variance (ms^2)	34.08 ± 40.64	31.01 ± 40.74	48.78 ± 85.40	24.34 ± 30.26	P = 0.8282
RMSSD (ms)	4.02 ± 2.85	5.12 ± 4.04	2.38 ± 1.33	3.62 ± 3.17	P = 0.3537
Frequency domain (Mean ± SD)					
Very low frequency (%)	16.13 ± 15.72	7.375 ± 7.170	13.25 ± 13.30	15.13 ± 11.32	P = 0.4977
Low frequency (%)	19.00 ± 13.97	30.75 ± 18.53	10.88 ± 4.55[b]	10.88 ± 4.19[b]	**P = 0.0073**
High frequency (%)	65.00 ± 25.52	61.38 ± 18.61	75.38 ± 17.34	74.13 ± 13.78	P = 0.4026

Data correspond to measurements obtained from 8 animals in each group. SD: Standard deviation. ANOVA: One way analysis of variance. *Mean, standard deviation and variance of pulse interval. RMSSD: Root mean square of the successive differences. The letters [a](P<0.01) and [b](P<0.05) denote statistically significant differences compared to ISO group (Tukey's test).

exert their effects on the cardiovascular system is an expanding area with great potential for the discovery of new molecules (Tirapelli et al. 2010).

There was not a statistically significant difference between the SBP of the four groups, and in addition, the ISO and EOAz + ISO groups presented a non-significant drop in SBP. This result was not expected since previous studies showed an antihypertensive effect of EOAz, however, such studies were performed using hypertension models (Cunha et al. 2013, Lahlou et al. 2003), or in normotensive rats with intravenous EOAz (Lahlou et al. 2003). Some studies have shown that MI is related to hypotension, both in humans (Grassi & Mancia 1994, Kala et al. 2017) and in animals (Meyrelles et al. 1994).

In the HR analysis, we observed a decreased HR in the ISO group when compared to the EOAz group. On one hand, this result was not expected, since in previous studies, a negative chronotropic effect of EOAz was observed in isolated atrium (Santos et al. 2011) and anesthetized normotensive mice when administered EOAz in bolus (Lahlou et al. 2002). However, there are no published studies evaluating the effect of EOAz administed by gavage on the HR of normal rats or rats with MI. On the other hand, studies with a MI model show that in this type of lesion HR of the animals decreases when compared to control animals (Meyrelles et al. 1994). A statistically significant difference in double product was observed between the four groups. This difference is probably mainly due to HR, since this was increased in the EOAz group animals and there were no significant differences in SBP. RPP is a noninvasive parameter that reflects myocardial oxygen consumption, and a

Table IV. Blood pressure variability parameters at the time and frequency domain.

Parameter*	Control	ISO	EOAz	EOAz+ISO	Significance (ANOVA)
Time domain (Mean ± SD)					
Mean (mmHg)	117.46 ± 15.67	101.07 ± 7.20	113.64 ± 29.12	92.52 ± 19.17	P = 0.0590
Standard deviation (mmHg)	3.73 ± 2.58	2.31 ± 0.70	3.73 ± 2.22	2.25 ± 0.95	P = 0.1818
Variance (mmHg)	19.74 ± 29.68	5.758 ± 3.41	18.22 ± 22.66	5.83 ± 4.92	P = 0.2921
Frequency domain (Mean ± SD)					
Very low frequency (%)	21.63 ± 16.52	12.38 ± 11.07	13.25 ± 7.686	24.38 ± 13.64	P = 0.1705
Low frequency (%)	18.00 ± 15.00	35.50 ± 29.30	7.75 ± 5.47[a]	13.38 ± 14.06	**P = 0.0282**
High frequency (%)	60.38 ± 27.07	44.63 ± 25.29	79.25 ± 9.74[a]	62.25 ± 23.94	**P = 0.0407**

Data correspond to measurements obtained from 8 animals in each group. SD: Standard deviation. ANOVA: One way analysis of variance. *Mean, standard deviation and variance of systolic blood pressure. The letter [a](P<0.05) denote statistically significant difference compared to ISO group (Tukey's test).

decrease in this parameter is related to a lower risk of cardiovascular problems (Fornitano & Godoy 2006).

In addition to the electrocardiographic parameters, the data found in infarcted animals were consistent with data previously reported in other studies, with the exception of the QRS complex (Aman et al. 2012, Yousefi et al. 2013). The QRS complex was shown to be larger in the infarcted groups (ISO and EOAz + ISO), a result contradictory to other studies that showed a decrease of this parameter in infarcted animals (Aman et al. 2012). An enlargement of the QTc interval was observed, probably due to an increase (El-Marasy et al. 2020). An elevation of ST was also observed in ISO and EOAz + ISO groups, one of the most important electrocardiographic findings of the acute phase of myocardial infarction (El-Marasy et al. 2020). Thus, it can be observed that EOAz did not reduce the electrocardiographic changes caused by isoproterenol.

The study of cardiovascular variability in humans with heart failure has revealed the enormous potential of variability parameters as predictors of morbidity and mortality, as well as for patient prognosis (Lombardi et al. 1987, Spallone 2018).

Analysis HRV parameters in the frequency domain, showed a significant reduction in the percentage of LF components in the EOAz and EOAz + ISO groups when compared to the ISO group. It was also observed that the percentages in the EOAz and EOAz + ISO groups were smaller than in the Control group, and that the ISO group had a higher LF percentage than the Control group, although these differences were not significant. The HF band corresponds to a vagal

Table V. Baroreflex sensibility evaluation (reflex bradycardia and tachycardia).

Parameter	Control Mean ± SD	ISO Mean ± SD	EOAz Mean ± SD	EOAz+ISO Mean ± SD	Significance (ANOVA)
Bradycardic baroreflex (bpm/mmHg)	-0.54 ± 1.34	-2.54 ± 5.08	-0.911 ± 1.20	-1.35 ± 2,53	P = 0.5712
Tachycardic baroreflex (bpm/mmHg)	-0.38 ± 0.17	-0.14 ± 0.24	-0.23 ± 0.25	-0.22 ± 0.20	P = 0.1853

Data correspond to measurements obtained from 8 animals in each group. SD: Standard deviation; ANOVA: One way analysis of variance.

Table VI. Mean blood pressure and heart rate variation induced by stimulation of cardiopulmonary reflex (Bezold-Jarisch) by serotonin infusion.

Serotonin doses	Control Mean ± SD	ISO Mean ± SD	EOAz Mean ± SD	EOAz+ISO Mean ± SD	Significance (ANOVA1)
Mean blood pressure variation					
2 µg/kg	-33.08 ± 16.06 (n = 7)	-20.24 ± 12.49 (n = 8)	-37.34 ± 22.03 (n = 8)	-34.39 ± 13.16 (n = 8)	P = 0.1906
4 µg/kg	-21.25 ± 21.09 (n = 7)	-6.47 ± 3.74 (n = 8)	-16.65 ± 9.65[a] (n = 7)	-13.15 ± 8.91[c] (n = 8)	P = 0.1452
16 µg/kg	-24.71 ± 14.96 (n = 7)	-9.09 ± 11.52 (n = 8)	-16.49 ± 11.23[a] (n = 7)	-9.66 ± 6.16[c] (n = 8)	**P = 0.0439***
Significance (ANOVA2)	P = 0.2641	P = 0.0626	**P = 0.0076**	**P < 0.0001**	
Heart rate variation					
2 µg/kg	-9.32 ± 25.46 (n = 7)	-0.10 ± 4.64 (n = 8)	0.81 ± 17.58 (n = 8)	-6.37 ± 21.90 (n = 8)	P = 0.6775
4 µg/kg	-6.18 ± 19.49 (n = 7)	-8.67 ± 15.41 (n = 8)	-15.89 ± 11.80 (n = 7)	1.27 ± 4.03 (n = 8)	P = 0.1393
16 µg/kg	-44.29 ± 28.86[a,d] (n = 7)	-18.45 ± 21.83[a] (n = 8)	-48.34 ± 28.29[b] (n = 7)	-9.49 ± 8.78[e,f] (n = 8)	**P = 0.0061**
Significance (ANOVA2)	**P = 0.0047**	**P = 0.0379**	**P = 0.0063**	P = 0.3101	

SD: Standard deviation. ANOVA: One way analysis of variance. The letters [a](P<0.05), [b](P<0.01) and [c](P < 0.001) denote statistically significant differences compared to 2 µg/kg at the same group, and [d](P < 0,01) denote differences compared to 4 µg/kg at the same group. [e](P<0.05) and [f](P<0.05) denote statistically significant differences compared with Control and EOAz groups, respectively (Tukey's test). * indicates significancy for ANOVA but not for Tukey's test.

activation indicator, whereas the LF component is due to joint action of the sympathetic and parasympathetic systems (Khodadadi et al. 2020, Meister et al. 2019), with a predominance of the sympathetic system. Thus, the decrease of the LF component, associated with a tendency of decrease in the LF / HF ratio in the EOAz and EOAz + ISO groups, suggests a decrease in the action of the sympathetic nervous system on the heart in the groups treated with EOAz. Signs of exacerbated sympathetic activation are often observed in patients with MI (Lombardi et al. 1987). It has been observed that an increase in sympathetic action on the heart increases mortality in MI patients and mice (Ziegler et al. 2018). Therefore, pre-treatment with EOAz possibly decreases the risk after MI because it causes a decrease in sympathetic modulation

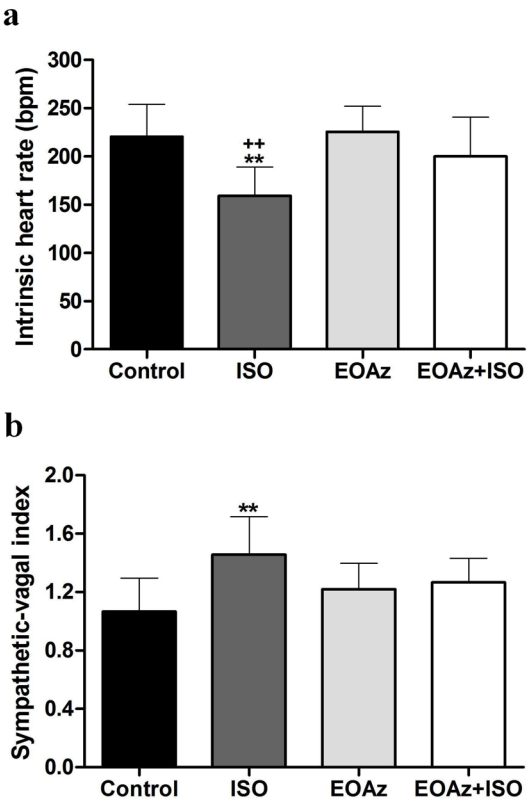

Figure 2. Intrinsic heart rate (IHR), in beats per minute (bpm) (a) and sympathetic-vagal index (b). Data expressed as mean and standard deviation in, respectively, 7, 8, 6 and 8 animals from Control, ISO, EOAz and EOAz+ISO groups. **P<0.01 compared to Control group; ++P<0.01 compared to EOAz group.

on the heart. Systolic blood pressure variability (SBPV), showed a significant decrease in the percentage of LF component in the EOAz group, in addition to an increase in the value of the HF component, when compared to the ISO group.

The baroreceptor or baroreflex, one of the most important mechanisms for the beat-to-beat control of blood pressure, acts to adjust heart rate and vascular sympathetic activity moment-by-moment (Abdulla et al. 2019, Su et al. 2002). Clinical studies have demonstrated that baroreflex impairment is an independent risk factor for sudden death in post MI patients (La Rovere et al. 1998).

In relation to cardiopulmonary reflex, the present study demonstrated a greater drop in BP of the EOAz + ISO group compared to the ISO group, and an increase in HR in these same groups (EOAz + ISO and ISO), when serotonin doses were administered. Situations that cause changes in the central blood volume or subatmospheric pressures in the lower part of the human body activate the cardiopulmonary reflexes (Meyrelles et al. 1994). This cardiopulmonary reflex is known as the Bezold-Jarisch reflex, which results in increased vagal activity and reduced sympathetic activity (Meyrelles et al. 1994, Vasquez 1994).

In pioneering studies evaluating the cardiopulmonary reflex in male rats submitted to MI, a reduction in the sensitivity of the cardiopulmonary reflex caused by ligation of the left coronary artery was observed (Aires et al. 2017, Vasquez 1994). This reflex cardioinhibitory dysfunction represents the consequences of the progressive morphofunctional changes of the cardiac muscle after MI, probably associated with inactivation of the receptors in the MI area and hypertrophy of the remaining left ventricle area (Meyrelles et al. 1994, Vasquez 1994). In another study, a reduction in cardiopulmonary reflex was also observed in rats with isoproterenol induced (treated for 15 days with isoproterenol) MI. It is consensus that cardiopulmonary reflex is reduced in infarcted animals has not been reached because an increase in hypotensive and bradycardic responses was observed in a study with infarcted rabbits (Vasquez 1994).

IHR was significantly lower in the ISO group compared to the Control and EOAz groups, but was not lower in the EOAz + ISO group, evidencing a decrease in intrinsic heart automatism in MI (Krüger et al. 2000).

Several studies have shown that in MI there is a decrease in parasympathetic modulation and an increase in sympathetic modulation. In healthy individuals, situations of increased sympathetic activity are often concomitant

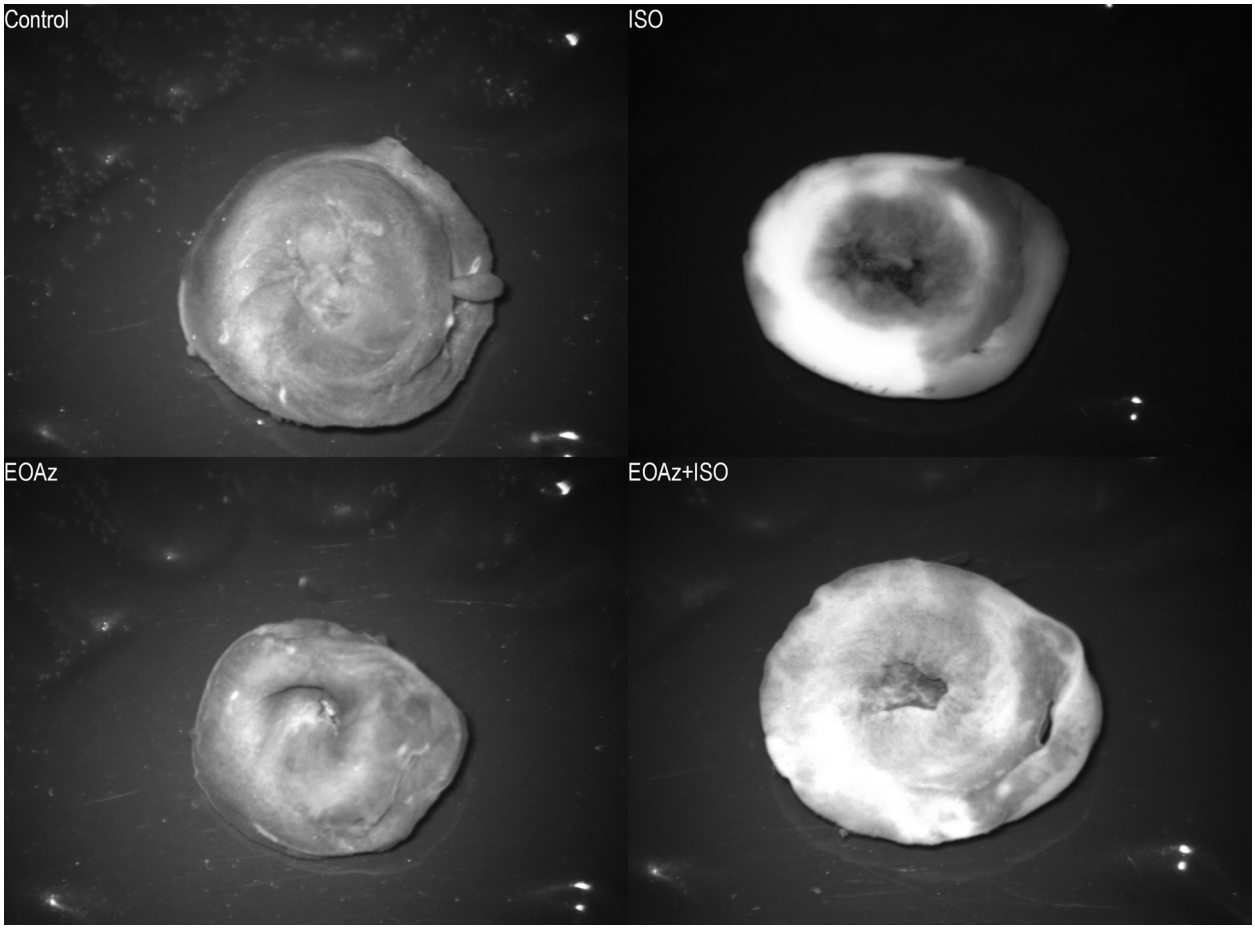

Figure 3. Rat heart sections TTC-stained. Infarcted areas, shown as pale area, are more condensed in ISO group, ant more diffused in EOAz+ISO group. Control and EOAz groups without infarcted areas.

with an increased LF band. In clinical and experimental studies of heart failure, increases in the LF band of HRV are related to the degree of sympathetic excitation, evaluated by direct measures of sympathetic nerve activity or plasma norepinephrine (La Rovere 2003).

In this study, there was a significant increase in the SVI in the ISO group compared to the Control group and, although not statistically significant, the mean values of this index were also higher in the ISO group compared to the EOAz and ISO + EOAz groups (SVI> 1 = sympathetic predominance, SVI <1 = parasympathetic predominance). These values suggest that pre-treatment with EOAz was able to decrease sympathetic modulation on the heart, thus contributing to a positive prognosis after MI. These results are similar to previous studies (Krüger et al. 1997, 2000) and indicate that in the model of moderate heart failure after MI in rats, there is a loss of vagal parasympathetic tonus on the heart, with the sympathetic tonic action remaining intact, directed to the sinus node. There are several reports in the literature stating that increased cardiac sympathetic activity after MI is one of the factors closely related to morbidity and mortality, in addition to generating baroreflex dysfunction in patients with MI (Krüger et al. 2000).

When stained with TTC, the heart sections of the Control and EOAz groups did not show ischemic (pale) areas. In the ISO group the

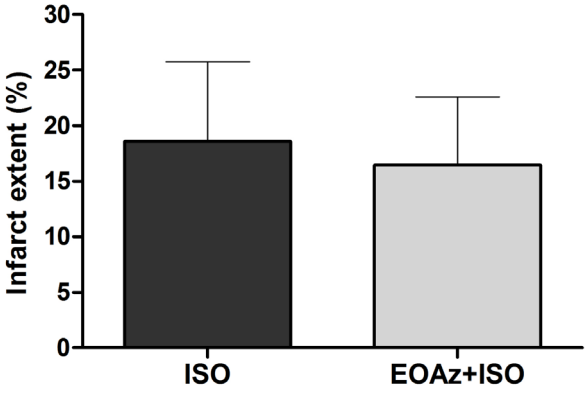

Figure 4. Infarct extent evaluation, expressed in percent, in animals from ISO and EOAz+ISO groups. The infarct extent was evaluated by TTC method contrasting the infarcted area. Data expressed as mean and standard deviation from measurement performed in 8 animals from each group.

infarcted area was well defined and localized, unlike the EOAz + ISO group where such areas were diffuse. Using the same technique, but in an anterior descending coronary artery ligation MI model, Almeida et al. (2009) showed there was no significant reduction of the infarct area in animals treated with angiotensin converting enzyme inhibitors. In a model of isoproterenol infarction, Polo in 2014 (unpublished data), observed no reduction of infarct area in animals submitted to pre-treatment with essential oil of *Citrus aurantium L.* Asanuma et al. (2004) showed that infusion of carvedilol reduced the infarct area in dogs undergoing ischemia followed by reperfusion, however Dantas et al. (2013) did not detect this reduction with acute and chronic infusion of carvedilol in the MI by ligature of the left coronary artery model. The different methodologies and techniques used in these studies, coupled with the variation in substances tested, make it difficult to compare studies with our finds and form conclusions regarding the mechanism of cardioprotection in the extent of the infarct area.

EOAz was not effective in reversing the changes in cardiovascular and electrocardiographic parameters caused by AMI. However, in relation to the autonomic parameters, a reduction in cardiac sympathetic modulation was observed in animals pretreated with EOAz. Therefore, it is possible that EOAz has a role preventing the occurrence of probable complications concerning to autonomic modulation dysfunction after myocardial infarction.

CONCLUSION

Preliminary treatment with the essential oil of *Alpinia zerumbet* was not able to reverse changes in hemodynamic or electrocardiographic parameters caused by acute myocardial infarction. However, there was a reduction in cardiac sympathetic modulation, suggesting that in this aspect, the essential oil of *Alpinia zerumbet* has a possible role in reducing the dysfunctional autonomic modulation after myocardial infarction.

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