Full Length Research Paper

Protective effect of commercial extracts of oregano (Lippia graveolens) against cardiac ischemia-reperfusion damage

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Cardiac ischemia includes severe damage to heart muscle tissue due to decreased or cessation of blood flow, which involves failure to supply nutrients and oxygenation to heart cells. Causes can include blockages due to fat and cholesterol deposits. These damages include cell apoptosis, calcium and sodium concentration imbalance, loss of contraction force, and formation of reactive oxygen species. In the present work, oregano extracts administered to Wistar rats were tested. After 15 days of treatment, the hearts were extracted and the pharmacological response was evaluated in Langendorff isolated perfused heart assay. Left ventricular contractility and perfusion pressure were evaluated before and after inducing an ischemia-reperfusion event by administering three different drugs to stimulate said response. The results, expressed as a percentage of loss of cardiac functionality, suggest a protective effect against cardiac damage in those hearts treated with the extracts after each event.

Key words: Wistar rats, cardiac ischemia, Langendorff, oregano extracts.

INTRODUCTION

The heart is a hollow organ made up of two atria and two ventricles that work rhythmically to distribute blood in the body at three levels: systemic, pulmonary, and the coronary area. Blood reaches the heart through the atria and is propelled through the ventricles. The movement of the atria and ventricles is done in an orderly and coordinated way, in a cycle that repeats itself over and over with each beat and is known as the cardiac cycle (Adeyene et al., 2006). The distribution of blood in the body generates a continuous supply of oxygen, reduces
the amount of carbon dioxide, helps to eliminate waste substances, distributes nutrients and physiological substances to body tissues, among others. The foregoing shows that any damage that is generated to the heart represents a negative effect on the quality of life of the individual (Ganty and Suzuki, 2012).

Cardiovascular disease is the leading cause of death and disability in the world, with an economic burden of $47 trillion for the next 25 years. Although the global incidence of hypertension, smoking and hypercholesterolemia has decreased, obesity, diabetes and aging have increased dramatically in the world and with it the possibility of suffering from any of these cardiovascular diseases (Conti, 2010; Faruque et al., 2018). In 2000, there were 2 million deaths from this cause, but by 2019, the number was almost 9 million, which represents 16% of deaths in the world (WHO, 2020). Ischemia (I) is defined as a series of damages to various cells and functions due to partial or total obstruction of blood flow, which includes restriction of the supply of nutrients and oxygen (Buja, 2005). Unfortunately, about 50% of cases are fatal in the first 5 years after diagnosis. Among the main risks factors is high blood pressure, obesity and smoking (Bryce-Moncloa, 2017).

The most severe damage from ischemic events is the death of myocytes. Reperfusion is the event of restoration of blood flow. Both events (I-R) can damage cardiac tissue to different degrees depending on the duration of the event, intensity of reperfusion, calcium overload, mitochondrial permeabilization, formation of reactive oxygen species (ROS) and pro-inflammatory process. In prolonged I-R events, myocytes are highly damaged and induced to apoptosis and lysis, especially at the beginning of reperfusion, leading to sarcolemmal rupture, mitochondrial edema and massive calcium deposits (Ruiz and Garcia, 2009).

The first condition during ischemia is hypoxia, which forces myocytes to carry out an accelerated glycolysis process anaerobically, producing large amounts of lactic acid. This starts a cascade of biochemical signaling. As a consequence of the increase in H + ions, the cell introduces Na + by activation of sarcolemmal transporters. This accumulation of Na + considerably damages the contractile capacity of myocytes, (Abad et al., 2018). To eliminate Na +, another transporter (Na / K) is activated, which favors the entry of large amounts of Ca + and the expulsion of Na +. The sarcoplasmic reticulum, which is the normal Ca + reservoir, cannot capture excess calcium as it requires ATP for its function, but ATP is scarce. This condition now activates other enzymes such as nucleases, phospholipases and proteases that cause the destruction of the cell membrane and therefore, the death of the cell (Luna et al., 2003; Anja et al., 2012). The main damage to cardiac tissue during I-R events is loss of muscle contractile capacity.

Torres et al. (2018) successfully tested the use of *Matricaria recutita* (chamomile) in I-R events. They used hydro-alcoholic extracts in Wistar rats measuring vascular and ventricular contractility before and after ischemia. Similar results are reported by García et al. (2014) when treating Wistar rats with extracts of chaya (*Cnidoscolus chayamansa*). So, it is clear that some plants can have a beneficial effect on the heart, however, there are few reports about oregano and its effects in I-R events. The aim of the present study was to evaluate the cardio-protective effect of commercial extracts of oregano (*Lippia graveolens*) in I-R events in a Langendorff isolated heart model with Wistar rats.

There are also some antecedents that suggest the beneficial effect of the compounds present in oregano. Canbek et al. (2007) directly tested carvacrol, which is one of the major compounds present in oregano, in liver ischemic events in Wistar rats with good results. Azari et al. (2017) administered oregano extracts orally for one week to rats that were then subjected to intestinal ischemia. The subsequent histopathological study of the jejunum showed minor damage compared to the untreated groups. Guan et al. (2019) also report the protective effect of carvacrol in cerebral ischemia events. Despite all these reports, no antecedents were found to evaluate, in specific, the protective effect of oregano extracts in myocardial ischemia.

**MATERIALS AND METHODS**

**Samples and extracts**

Commercial extracts manufactured by “Integradora de Productos y Servicios Oregánico” enterprise were used. This company is a cooperative in the town of Mezquital, Durango, Mexico (23° 28′ 22 ″ N 104° 24′ 40 ″ W) with a majority indigenous population. The oregano harvest is done manually in the mountainous areas of the region. The extracts are obtained by distillation by means of "steam stripping" obtaining the concentrated essential oils. These oils are then diluted in olive oil 1:50 ratio and packaged for sale and consumption.

**Bio-models**

Wistar rats were acquired at the Biosciences Center of the Autonomous University of San Luis Potosí, Mex., weighing 120-150 g. The rats had *ad libitum* access to food and water. All animals were treated with care according to the guidelines established by the Mexican standard NOM-062-ZOO-1999, which specifies the care that must be taken in laboratory animals.

**Treatments**

Rats were randomly divided into three groups of six rats each. One group was given 500 µl of oregano extract via cannula daily. The second group was given the same amount of olive oil as the first control, because the extracts are diluted in this oil. Finally, the third group was given saline solution and also served as a control group. All treatments lasted 15 days prior to the trial.
Ischemia assay

Langendorff isolated perfused heart assay: At the end of the treatment period, each rat was sedated and the heart was removed which was mounted on a Langendorff equipment. Despite its age, the Langendorff isolated perfused heart assay continue to present great advantages in the study of various heart conditions, both in mice and in other species. Advantages include the administration of drugs to assess responses, measure contractility functionality, ventricular functions and perfusion pressure (Hong et al., 2018). Each heart was supplied with a Krebs-Henseleit solution (115 mM NaCl, 4.6 mM KCl, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, 2.5 mM CaCl$_2$, 25 mM NaHCO$_3$ and 11 mM glucose) mixed with 95% O$_2$ and 5% CO$_2$ with a flow of 10 ml / min. The pH was kept at 7.4 and the temperature was kept at 37°C, (Antilla et al., 2017). Before starting assay, it was allowed to stabilize for ten minutes.

To analyze the effect of the extracts, the pharmacological response of endothelial functions (perfusion pressure) and contractile functions of myocytes (left ventricular contractility) was evaluated. 20 µl of each drug tested was administered separately at 1 µM concentration. The drugs that were supplied: Phenylephrine, which is a vasoconstrictor (α-1 receptor agonist, which stimulates the activity of phospholipase C involved in signal transduction); Angiotensin II, which is a set of peptide hormones derived from angiotensinogen, which cause vasoconstriction and a subsequent increase in blood pressure and increased contractility; and Acetylcholine that produces vasodilation, decreased heart rate (negative chronotropic effect), decreased conduction velocity of the sinoatrial and atrioventricular node and a decrease in the force of cardiac contraction (negative inotropic effect). These three drugs are chosen because the stimulus they exert on the heart has been widely demonstrated. The first drug was administered and the cardiac response was evaluated. Ischemia then occurred due to the cessation of the flow of the Krebs-Henseleit solution for 20 min. The flow was immediately re-established, the same drug was administered and cardiac function was evaluated again. The same was done with each of the other two drugs. The cardiac response before and after of the Ischemia-Reperfusion (I-R) event was compared. Before ischemia, the cardiac response was taken as 100% of functionality, and the response after the I-R event was taken as the basis for calculating the loss of functionality. The results are expressed as a percentage of loss of left ventricular function and as a percentage of loss of perfusion pressure with each of the drugs. To compare the groups, an ANOVA and the Bonferroni post hoc test were applied using IBM SPSS Statistics 22 Software.

RESULTS

As shown in Figure 1, loss of cardiac function after ischemia, in terms of perfusion pressure, was markedly higher in the hearts of untreated rats (control group) with phenylephrine stimulus. The hearts of the rats treated with oregano showed the lowest loss of functionality (almost half that of the control group with the same phenylephrine). The same condition can be seen with the stimulus of angiotensin. In both cases, the ANOVA test and the post hoc test shows the oregano and control groups present a statistically significant difference (p<0.05). On the other hand, although olive oil does not present a significant difference in relation to oregano group, it can be seen that the group treated with oregano presents less loss of functionality.

Regarding the loss of functionality of the left ventricle, the results are shown in Figure 2. Less loss is seen in those hearts treated with oregano extract before the three drugs compared to the hearts of the control group (without treatment). Likewise, for the effect of olive oil, a greater loss of functionality of the left ventricle is observed compared to those treated with oregano, when stimulated by acetylcholine and angiotensin. The ANOVA
Figure 2. Percentage of loss of cardiac function. Left ventricular function.

test and the post hoc test showed significant differences (p<0.05) between the control and oregano groups with acetylcholine and angiotensin stimuli.

**DISCUSSION**

In general, it can be pointed out that the protective effect of oregano in relation to the control group is evident in 4 of the 6 groups studied: Perfusion pressure with phenylephrine and angiotensin and ventricular function with acetylcholine and angiotensin. On the other hand, the effect of olive oil seems to be also good, however, the differences in three of the 6 tests between oil and oregano show that oregano could, by itself, present a protective effect against the events of ischemia reperfusion. Cota et al. (2018) induced myocardial infarction in rats. One group of these rats was previously treated with oil of oregano and showed considerably less damage to the myocytes. Similar to our trial, the authors found that the contraction force of myocytes decreased little in the oil-treated groups. The authors attribute this effect to the presence of phenolic compounds, carvacrol and thymol, which are widely known to be present in oregano. It appears that oregano essential oil maintains the original structure of myocytes by inhibiting the release of protease or apoptosis-inducing enzymes. Similar observations are made by González et al. (2018) when reviewing various works that attribute a protective effect against ischemia in polyphenolic extracts of various plants.

Another aspect by which the heart can lose contractility during ischemia is the formation of reactive oxygen species. During the hypoxia produced by ischemia, the calcium concentration increases causing a series of changes, among them, the transformation of the dehydrogenase enzyme to xanthine oxidase, producing urates and the formation of superoxide and peroxide radicals (Abad et al., 2018). In 2017, Chen et al, demonstrated that carvacrol increased the activity of the enzymes superoxide dismutase and catalas, which act on the superoxide and peroxide species, thereby reducing the damage due to ischemic events in rats previously treated with carvacrol.

NF-kb promoting the formation of pro-inflammatory proteins such as IL-6 and TNF-a (Abad et al., 2018). In the same work by Chen (2017) they show that the presence of carvacrol decreases the presence of ROS due to the increase of the superoxide-dismutase enzyme and the decrease of malonaldehyde. They also showed that carvacrol can favorably regulate the permeability of mitochondria by reducing the excess of Ca++, which would also mean a decrease in the formation of ROS. Both the formation of ROS and the expression of pro-inflammatory proteins and necrotic processes are diminished by the administration of carvacrol. The low loss of cardiac functionality in our experiment in the group treated with extracts could be explained by the presence of compounds such as carvacrol and thymol in the extracts tested. Although there are several reports about the protective effect of olive oil in ischemic events, mainly attributed to fatty acids such as ω3 and phenolic compounds (Gonzalo et al., 2019; Bukhari et al., 2020), the works revolve around cerebral ischemia. In our case,
the olive oil had a good protective effect, but the combined extracts seem to have a better performance.

**Conclusion**

The responses to pharmacological stimuli of rat hearts previously treated with oregano extracts suggest a protective effect in ischemia-reperfusion events. Although the olive oil present in the extracts also has a good effect, it seems that the oregano extract, by itself, provides a protective effect independent of ischemic events. It seems that the presence of compounds such as carvacrol and thymol in the extracts reduces the damage to the heart tissue and protects its contractile function. Frequent use of the extracts is recommended.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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