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Anti-Inflammatory Effect Of Mastruz (*Chenopodium Ambrosioides)* Extract In Respiratory Distress Syndrome

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Abstract: *Acute Respiratory Distress Syndrome (ARDS) is a respiratory failure acute onset, with bilateral pulmonary infiltrate on chest X-ray and diffuse alveolar damage to pathology, developing non-cardiogenic pulmonary edema. Chenopodium ambrosioides known for its healing effect, anti-asthmatic, anti-inflammatory and respiratory stimulant. The objective is to analyze the improvement of respiratory function with inhalation of Chenopodium ambrosioides extract. Fifteen rats were used of the species Rattus norvegicus. The animals were divided into 3 groups of 5 animals each, the sham group, treated group and the false-operated group. ARDS was induced by the ischemia-reperfusion method of the mesenteric artery in the first two groups, with subsequent inhalation of alcoholic extract of mastruz by the treated group. After 90 minutes, the rats were euthanized and the lungs were removed for histological and morphological analysis. Treatment with alcoholic extract of mastruz led to decreased lung inflammation induced by I/R, since the group treated with inhalation of the extract showed less inflammatory cells in the lung parenchyma compared to the sham group (p <0.05). It is suggested that the alcoholic extract of mastruz reduces the pulmonary inflammatory response and consequently the syndrome of acute respiratory distress arising from the systemic action induced by I / R.*
Keywords: *Acute Respiratory Distress Syndrome, mastruz, anti-inflammatory*

I. Introduction

The Syndrome of Acute Respiratory Distress (ARDS) is an inflammatory process of pulmonary edema, acute, non-hydrostatic or non-cardiogenic, accompanied by a persistent and severe hypoxemia, defined as PaO 2 / FIO 2 ≤ 200 (ANTONIAZZI et al, 1998) and pressure occlusion of the pulmonary artery ≤ 18 mmHg (BERNARD et al, 1994). Costa et al. (1991) and Pinheiro et al. (2007) describe four clinical stages of development having as initial symptoms dyspnea and tachypnea with normal chest X-ray, causing high mortality rates that reach 60% and prolonged stay in ICU survivors.

The incidence of the syndrome vary among authors, being estimated between 1.5 and 75 cases per 100,000 inhabitants, being lower in childhood - from 2.6 to 12.8 cases per 100 thousand inhabitants - and most at the age of 75 at 84 - 306 cases per 100 thousand inhabitants (PINHEIRO; OLIVEIRA, 2004; BARBOSA; BARBOSA, ROCCO, 2011; AMATO et al., 2007).

Barbas and Matos (2011) report that ARDS is the most severe spectrum acute lung injury (ALI) is characterized pathologically by diffuse alveolar damage, with the pathophysiology of the development of non-cardiogenic pulmonary edema. Such swelling is due to increased permeability of the pulmonary alveolar-capillary membrane providing extravasation of fluid rich in proteins into the alveolar space causing an injury to the alveolar-capillary membrane.

Its pathogenesis is two-way, the first is from the direct effects of an injury in the lung cells, the second is through an acute systemic inflammatory response that may include cellular and humoral components. The risk factors most commonly related to ARDS are: by direct injury (aspiration, pulmonary infection, drowning, toxic inhalation, pulmonary contusion fat embolism, oxygen toxicity) or indirect injury (sepsis syndrome, multiple trauma, multiple transfusions, shock, large burns, pancreatitis, "By-pass", cardiopulmonary, exogenous intoxication, disseminated intravascular coagulation, excess fluid) (ANTONIAZZI et al, 1998; Pinheiro; OLIVEIRA, 2004; GARCIA, PELOSI, 2011). For Barbas and Matos (2011) handling and treatment of patients with ARDS is a constant challenge, and of fundamental importance to identify the etiologic agent because, with the elimination of the cause, you can stop the natural history of the disease. Costa et al. (1991) reported that treatment of already installed ARDS consists mainly in medium: control and prevention of sepsis, greater fluid control to reduce edema, monitoring the pressure in the pulmonary circulation, and especially for the treatment of respiratory failure artificial ventilation.

The Chenopodium ambrosioides, known as Mastruz is an anti-inflammatory herbal and a respiratory stimulant widely used by common sense for the treatment of asthma, bronchial catarrh, bronchitis, cough, tuberculosis, lung infection, influenza and laryngitis (PLANTAMED, 2013).

Some pharmacological activities of Chenopodium ambrosioides are proven, such as antitumor, antifungal, healing of ulcers caused by Leishmania species, anti-inflammatory and analgesic (NASCIMENTO et al, 2006; JARDIM et al, 2008;. PATRICIO et al, 2008; IBIRONKE; AJIBOYE, 2007).

Given this proposed questioning about what the benefit of inhalation of mastruz extract (Chenopodium ambrosioides) in the treatment of Acute Respiratory Distress Syndrome in Rattus norvegicus.

This study aimed to analyze the improvement of respiratory function with inhalation of Chenopodium ambrosioides extract. The specific objectives proposed to verify the reduction of the inflammatory response of the respiratory epithelium affected by the syndrome with inhalation of Chenopodium ambrosioides extract (mastruz) and expand scientific knowledge about the respiratory effects of Chenopodium ambrosioides extract (mastruz).

II. Aims & Objectives

To analyze the improvement of respiratory function with inhalation of Chenopodium ambrosioides extract.

III. Material And Methods

3.1 Ethical Aspects

For the implementation of this study, we applied all the ethical principles of animal testing in accordance with the Federal Law No. 11,794 after approval of the research project by the Ethics Committee on Animal Use - CEUA the Faculdade Integral Diferencial – FACID DeVry, where the same was performed.

3.2 Search Type

The survey corresponds to an experimental study in rats, with a quantitative approach to the anti-inflammatory effect of Chenopodium ambrosioides extract (Mastruz) on acute lung inflammation in a model of ischemia and reperfusion.

3.3 Animals and Study Course

The study universe was composed of rats of the species Rattus norvegicus, Wistar (weighing 200-250g), females, reared in a private higher education institution, kept in temperature and humidity controlled and light-dark cycle of 12 hours starting at 6pm. The animals were kept in their homes cages (5/cage) with free access to water and food.

We used a total of 15 animals were randomly divided into three experimental groups of 5 animals each (Table 1).

Table 1: Distribution of the animals in the study

Groups	Experimental	N
I	SHAM (I-Ri)	5
II	Control (False-Operated)	5
III	Treated (I-Ri + Mastruz)	5

Legend: I-Ri = ischemia / reperfusion

3.4 Plant Material

The aqueous extract of mastruz was obtained from the fresh leaves of Chenopodium ambrosioides plant. This was taken at dawn the day and then taken to the physiology laboratory of a higher education institution, which we selected only the leaves estimating an approximate weight of 300 grams.

3.4.1 Preparation of the aqueous extract of mastruz

The mastruz leaves were washed in running water and then put to dry in the sunlight and at room temperature in 24 hours. Then the leaves were ground in a blender with 1 liter of absolute alcohol and 99% housed in a suitably sealed container and kept standing in a greenhouse for 24 hours.

After standing, the supernatant separated liquid being precipitated material that subsequently filtered with qualitative filter paper.

Initiated the extraction process rotary evaporator, remaining for a period of 24 hours where there was obtained mastruz extract and about 970 ml of absolute ethanol 99%. The extract was stored in amber vials of 30 ml for single use on the therapeutic intervention.

3.5 Surgical Induction of Acute Respiratory Distress Syndrome (ARDS)

The animals were pretreated with atropine 2% solution (0.2 ml for each animal weight of 100 g) and 15 minutes later anaesthetized receiving 0.1 ml for each animal weight of 100g composed of ketamine and 5% solution xylazine 2% in the ratio 1: 1, administered intramuscularly.

After the application of anesthetics, cited above, and after laparotomy and evisceration, intestinal ischemia was induced by clamping the superior mesenteric artery for 45 min.

The clamping vascular clamp was performed with micro-surgery. During the 45 minute period of ischemia, the abdominal incision remained covered with clear plastic to minimize loss of fluid and heat. After the desired period of ischemia, vascular clamp was removed, and start the reperfusion period.

3.6 Therapeutics

The therapy performed equivalent to nebulization with mastruz extract. After the ischemic time already described, this group was submitted to inhalation of 3 drops of mastruz extract for 2 minutes.

3.7 Euthanasia

After 45 minutes of reperfusion, animals were again anesthetized and sacrificed by overdose of anesthesia by the abdominal aorta after 90 minutes (Sham - ischemia and 90 min reperfusion, Treaty - ischemia and reperfusion mastruz extract + 90 min). Subsequently, the lungs were removed for histological analysis.

The group consisting of falsely operated animals (false-operated), wherein the abdominal incision was performed, but no ischemia was induced, were also sacrificed at 90 minutes and incorporated to the studies.

3.8 Histological Analysis of Lung

Lung histology was carried out in the systemic inflammation model. Therefore, the lungs were removed and the left lower lobe was separated and subjected to fixation and dehydration process. After routine laboratory procedure for making blades, the specimens were embedded in paraffin to allow microtmomia (with histological 3μm thick), followed by staining procedures with hematoxylin-eosin (HE) further samples lung tissues were microscopically examined in regard to inflammatory cells.

3.9 Analysis Morphometric

For morphometry, used a trinocular inverted microscope, mark LABOMED iVu3100, TCM 400 model coupled to a camera NA030 using PixelPro ™ software with acquisition of photos in 400X magnification. For each animal, they were obtained four images in different fields to carry out the count of the total number of inflammatory cells in the field of observation, using the computer Image J program in its function "cell contains."

3.10 Statistics

After data collection, they were organized in a spreadsheet in Microsoft Office Excel 2007. The statistical analyzes were conducted using the program GraphPad Prism 5.0. and subjected to analysis of variance (ANOVA) followed by Tukey test for comparison of means. The results were expressed as the mean ± standard error of the mean (SEM) and standard deviation (SD). P values <0.05 were considered significant.

IV. Results And Discussion

Some pharmacological activities of Chenopodium ambrosioides are proven, such as antitumor, antifungal, healing of ulcers caused by Leishmania species, anti-inflammatory and analgesic (NASCIMENTO et al, 2006; JARDIM et al, 2008; PATRICIO et al, 2008; IBIRONKE; AJIBOYE, 2007).

The pathogenesis of ARDS can be triggered in two ways, the first is from the direct effects of an injury in lung cells and another that is through an acute systemic inflammatory response that may include cellular and humoral components. The risk factors most commonly associated are: by direct injury (aspiration, pulmonary infection, drowning, toxic inhalation, pulmonary contusion fat embolism, oxygen toxicity) or indirect injury (sepsis syndrome, multiple trauma, multiple transfusions, shock, large burns, pancreatitis "By-pass", cardiopulmonary, exogenous intoxication, disseminated intravascular coagulation, excess fluid) (ANTONIAZZI et al, 1998; PINHEIRO; OLIVEIRA, 2004; GARCIA, PELOSI, 2011).

The interruption of the blood supply causes ischemic lesions which quickly generate active metabolites. Paradoxically, the restoration of blood flow to the ischemic tissue initiates a cascade of events that can lead to further cell damage known as ischemia reperfusion (I / R) injury that exceeds this initial ischemic insult. The restoration of blood supply, biochemical and molecular changes that occur during ischemia predisposes to damage mediated by free radicals (MALLICK et al., 2004; KÖHLER; DELUCCA; NETO, 2011).

According to Marqui et al. (2011) pulmonary microvasculature shown quite susceptible to the effects of activation of systemic inflammatory mediators resulting from oxidative stress in remote organs, giving theoretical support procedure performed in sham and treated groups, where the clamping of the superior mesenteric artery, followed by reestablishment of blood flow, was capable of triggering acute lung injury.

Arruda et al. (2006) observed an increase in serum TNF-alpha intestinal ischemia followed by reperfusion. For Antoniazzi et al. (1998) TNF-alpha is the most important cytokine in the pathogenesis of ARDS, since this, in addition to having a direct cytotoxic effect on the endothelium, also stimulates production of other cytokines such as the interleukins. There is an interaction of these inflammatory cells to vascular endothelium increased procoagulant activity and activation of neutrophils, monocytes and lymphocytes. These in turn release superoxide anions and other free radicals that participate in the endothelial tissue injury.

This process enables the onset of ARDS by indirect injury, corroborating the methodology applied in this study and the current results of the experimental groups in which they showed a cluster of inflammatory infiltrates.

It sought to determine the effect of inhalation of mastruz extract in preventing induced development of acute respiratory distress syndrome, since the plant is popularly used in inflammatory processes, anti-asthmatic and as a respiratory stimulant (PLANTAMED, 2013).

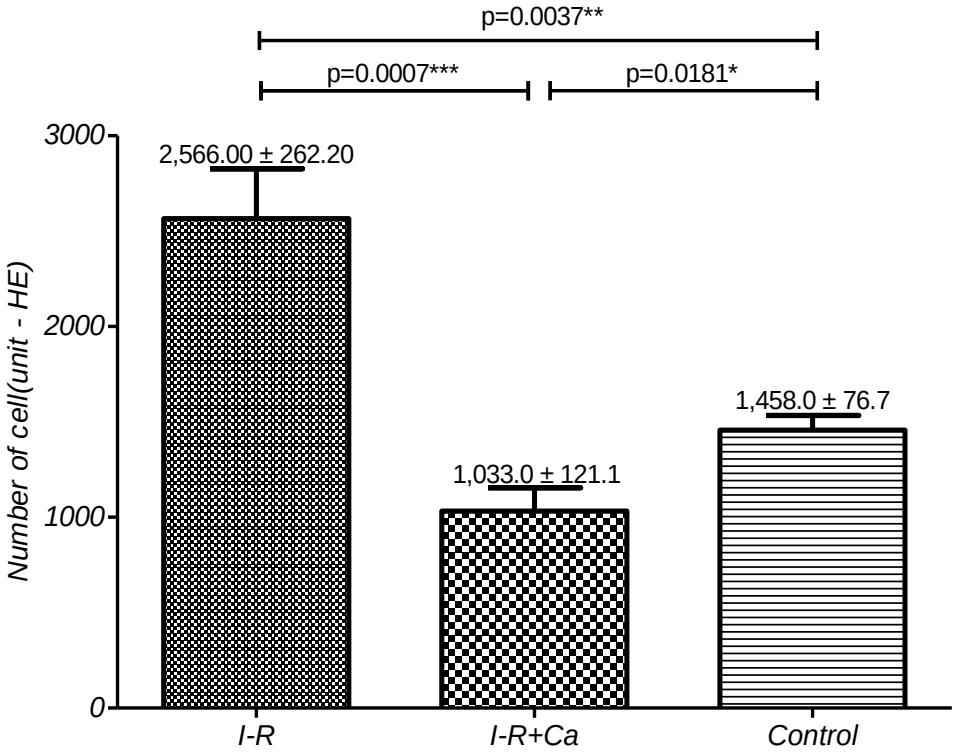

Figure 1: Number of inflammatory cells presented by study groups.

Legend: p for One-Way Test ANOVA post hoc Tukey test, with 95% and significance at p <0.05. Teresina, 2015. The results (Fig. 1) indicate that the lung tissue of animals subjected to treatment with nebulization of mastruz extract, with average values of 1033.0 (+ 121.1) along with the control group (false-operated) with average values of 1458.0 (+ 76.7), statistical significance of p <0.05 compared to group I-Ri (ischemia-reperfusion), with mean values of 2566.00 (+ 262.20), suggesting the reduction of inflammatory cells caused by ischemia and reperfusion and verifying the effectiveness of the extract effect on lung cells of rats.

The results showed that nebulised mastruz extract showed anti-inflammatory effect when administered three drops of the extract 2-minute period from 45 minutes after induction of lung inflammation, suggesting a modulation of pulmonary inflammatory response. It can be suggested that during some stage of the process of cell migration by reducing the number of inflammatory cells present in inflamed tissue, the release of cytokines and other inflammatory mediators are also reduced, thus enhancing the beneficial effects of mastruz extract.

In some works were used pharmacological models of inflammation (paw edema and ear) to assess the anti-inflammatory activity of the ethanol extract of the leaves and stems of C. ambrosioides and it could be verified that the extract administration was able to inhibit significantly edemas caused. When comparing the

effects of dexamethasone treatment with the extract was as effective to this drug, suggesting that the phytochemicals present could be causing inhibition of anti-inflammatory activity and edemigênica. The author of the study hypothesized that monoterpenes could be responsible for the observed effects (GRASSI, 2011).

Work in the same direction using methanol extract of leaves mastruz also demonstrated inhibition of the inflammatory process. The extract was responsible for the inhibition of certain mediators such as serotonin, histamine, substance P, bradykinin and prostaglandins (IBIRONKE; AJIBOYE, 2007).

Grassi (2011) sought a possible mechanism of action of the ethanol extract of mastruz through some inducing specific mediators of edema. It was found that the extract was able to reduce the edema induced by substance P (neurokinin involved in pain threshold and the associated inflammatory processes) and bradykinin (mediator of pain and inflammation). However, it was not possible to determine exactly what specific mechanism of action of the extract then being required more refined and specific studies.

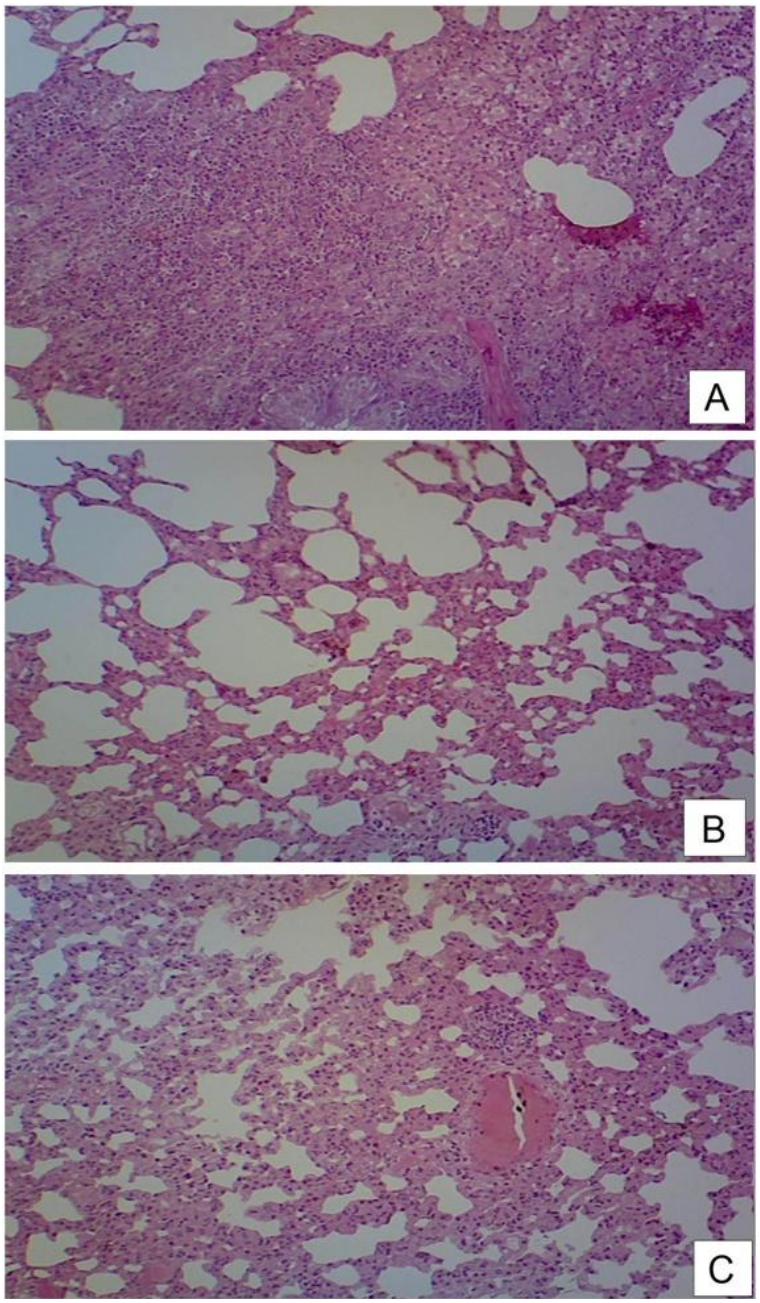

Figure 2: Microscopy of the left lower lobe. (A) sham; (B) treated with nebulization of mastruz extract; (C) false-operated.

Histological evaluation in a similar study sought a correlation between the degree of pulmonary involvement - through neutrophil count per field and the metabolic state in induced rat - and sepsis peritonitis, which can confirm the existence of systemic inflammatory response (ROCHA et al., 2007). The same

correlation was seen in this study, since there was an increase of inflammatory cells in the lung tissue of rats sham group compared to the control group (false-operated).

It is suggested that the incisional act in the abdominal region and the generation of free radicals triggered a parenchymal lung potential in the false group operated (control). A reasonable explanation for these results is the possibility of early depletion of lung antioxidant reserves in the face of the initial stimulus, but able to contain more pronounced damage (FERRO et al., 2010). This explains the presence, to a small degree, a greater number of inflammatory cells in the false-operated group compared to the group treated, even this having been subjected to ischemia and reperfusion, and not the first (Fig 1).

V. Conclusion

Based on the results obtained it can be concluded that treatment with the alcoholic extract of mastruz (Chenopodium ambrosioides) decreased significantly ($p < 0.01$) lung inflammatory response when compared with the group of rats which were subjected to I / R but who did not receive proper treatment.

It is proposed to perform more advanced and detailed studies of the possibility of using this extract as an alternative therapy to limit the syndrome acute respiratory distress as well as other local or systemic inflammation.

References

[1]. ANTONIAZZI, P. et al. Síndrome da Angústia Respiratória Aguda (SARA). Medicina, Ribeirão Preto, 1998. n. 31; pp. 493-506. Disponível em: <http://www.fmrp.usp.br/revista/1998/vol31n4/sindrome_angustia_respiratoria_aguda.pdf>. Acesso em: 05 de fev. de 2013.

[2]. BERNARD, G. R. et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med*, 1994; 149(3Pt1): pp. 818-24. Disponível em: <http://ajrccm.atsjournals.org/content/149/3/818.full.pdf>. Acesso em: 05 de fev. de 2013.

[3]. COSTA, V. R. et al. Tratamento alternativo na síndrome da angústia respiratória, no adulto: relato de caso. *Rev. Bras. Cir. Cardiovasc., vol 6; n.1; pp. 54-58*, 1991.

[4]. PINHEIRO, B. V. et al. Precisão do diagnóstico clínico da síndrome do desconforto respiratório agudo quando comparado a achados de necropsia. *J Bras Pneumol., 33(4):423-428*, 2007.

[5]. PINHEIRO, B. V.; OLIVEIRA J.C.A. SDRA - Síndrome do Desconforto Respiratório Agudo. Pneumoatual, 2004. Disponível em: <http://www.unifesp.br/dmed/pneumo/Dowload/Sindrome%20do%20Desconforto%20Pulmonar%20do%20Adulto.pdf>. Acesso em: 05 de fev. de 2013.

[6]. BARBOSA, M. C. M.; BARBOSA, A. P.; ROCCO, P. R. M. Diagnóstico da Síndrome do Desconforto Respiratório Agudo na Criança. *Pulmão RJ, vol.20, n.1, pp. 7-12*, 2011.

[7]. AMATO, M. B. P. et al. III Consenso Brasileiro de Ventilação Mecânica: Ventilação mecânica na lesão pulmonar aguda (LPA)/ Síndrome do desconforto respiratório agudo (SDRA). *J Bras Pneumol, 33 (Supl 2):S 119-S 127*, 2007.

[8]. GARCIA, S. N. B; PELOSI, P. Diferenças entre as Formas Pulmonares e Extrapulmonares da Síndrome do Desconforto Respiratório Agudo. *Pulmão RJ, 20(1):19-23*, 2011.

[9]. BARBAS, C. S. V.; MATOS, G. F. J. Síndrome do Desconforto Respiratório Agudo: Definição. *Pulmão RJ, vol.20, n.1, pp. 2-6, 2011*.

[10]. PLANTAMED. *Chenopodium ambrosioides* L. - ERVA-DE-SANTA-MARIA. 2013. Disponível em: <http://www.plantamed.com.br/plantaservas/especies/ Chenopodium_ambrosioides.htm>. Acesso em: 05 fev. 2013.

[11]. NASCIMENTO, F. R. F. et al. Ascitic and solid Ehrlich tumor inhibition by *Chenopodium ambrosioides* L. treatment. *Life Sciences, v.78, p. 2650 – 2653*, 2006.

[12]. JARDIM, C. M et al. Composition and antifungal activity of the essential oil of the Brazilian *Chenopodium ambrosioides* L. *Journal of Chemical Ecology, v.34, p. 1213-1218*, 2008.

[13]. PATRICIO, F. J. et al. Efficacy of the intralesional treatment with *Chenopodium ambrosioides* in the murine infection by *Leishmania amazonensis. Journal of Ethnopharmacology, v. 114, p. 313-319*, 2008.

[14]. IBIRONKE, G. F; AJIBOYE, K. I. Studies on the anti-inflammatory and analgesic properties of *Chenopodium ambrosioides* leaf extract in rats. *International Jounral of Pharmacology, v. 3, n. 1, p.111-115*, 2007.

[15]. MALLICK, I. H. et al. Ischemia–Reperfusion Injury of the Intestine and Protective Strategies Against Injury. *Digestive Diseases and Sciences, Vol. 49, No. 9 (September 2004), pp. 1359–1377*, 2004.

[16]. KÖHLER, F. H.; DeLUCCA, I. M. S; NETO, L. S. Antioxidantes enterais em lesões de isquemia e reperfusao em ratos. *Rev. Col. Bras. Cir. 2011; 38(6): 422-428*, 2011.

[17]. MARQUI, C. E. et al. Pretreatment with pentoxifylline attenuates lung injury induced by intestinal ischemia/reperfusion in rats. *Acta Cirúrgica Brasileira - Vol. 26 (6)* 2011.

[18]. ARRUDA, M. J. C. et al. Intestinal ischemia/reperfusion induces Bronchial hyperreactivity and increases Serum tnf-α in rats. *CLINICS ;61(1):21-8*, 2006.

[19]. GRASSI, L. T. *Chenopodium ambrosioides* L. – Erva de Santa Maria (Amaranthaceae): Estudo do potencial anti-inflamatório, antinociceptivo e cicatrizante. 2011. 147 f. Dissertação (Mestrado em Ciências Farmacêuticas) – Universidade Vale do Itajaí. Itajaí, 2011.

[20]. ROCHA, S. L. et al. Gasometria arterial e inflamação pulmonar de ratos com diferentes tempos de sepse abdominal. *ABCD Arq Bras Cir Dig ,20(1):28-33*, 2007.

[21]. FERRO, C. O. et al. Atividade da catalase no pulmão, rim e intestino delgado não isquemiado de ratos após reperfusão intestinal. *Rev. Col. Bras. Cir., 37(1): 031-038*, 2010.