Andiroba oil (*Carapa guianensis*) on ventral hernia repair

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In the Amazon rainforest region, Andiroba oil is an herbal oil that is widely used by the local population to treat several inflammatory diseases. The objective of this study was to test Andiroba oil as a mesh coating or by oral administration in a rat model of excisional abdominal wall defect as an alternative to modulate inflammatory response without impairing the ventral hernia repair. Thirty six animals were distributed into three groups (N=12). (1) In the control group (CONT), ventral hernia repair was done using polypropylene/polyglecaprone mesh. (2) In the Andiroba oil gavage group (AndG), animals were treated with the meshes and 0.63 ml/kg of Andiroba oil was given by oral administration for 7 days prior to the mesh placement. (3) In the Andiroba oil submersion group (AndS), animals were treated with meshes that were previously submersed in Andiroba oil. At the 7th, 14th, and 21st days, macroscopic and microscopic analyses were done. AndG had fewer adhesions, necrosis, and lymphocytes, as well as similar collagen fiber formation and fibrosis areas as CONT. AndS showed a higher number of macrophages, fibrosis area, and less collagen fiber formation. Oral administration of Andiroba oil modulated inflammatory response, reduced abdominal adhesion formation, and did not impair tissue healing.

**Key words:** Abdominal wall, wound healing, Andiroba, herbal, cicatrization.

**INTRODUCTION**

Incisional hernia is one of the most frequent complications of laparotomy; its incidence varies between 2 and 20% after all abdominal surgeries (Burger et al., 2006). However, actual incidence might be higher than the reported data, because most patients with incisional hernias have no symptoms. Hernia recurrence is one of the most undesirable complications, needing a complicated repair surgery and demanding high costs (Franklin et al., 2004). In the United States, the total number of ventral hernia repair (VHR) surgeries has increased substantially in the
past few years, having an annual cost of 3.2 billion dollars (Poulouse et al., 2012).

Incisional hernia treatment is essentially surgical and there are several techniques for their correction, as for example, primary suture of the wall (Baroncillo et al., 2008). However, the best results are obtained through mesh reinforcement, in which the chances of recurrence vary between 3 and 17%. It is clear that the use of meshes has revolutionized the outcomes of VHR (Montgomery, 2013).

Several mesh types have been widely used in surgical practice since their development. Meshes can be non-absorbable, absorbable, or partially absorbable; the last two being more commonly used (Pundek et al., 2010). Polypropylene, polyester, and ePTFE are the most commonly used meshes, and they also exhibit different properties. The development of new synthetic meshes is also extensive and fast (Montgomery, 2013).

The foreign body reaction is minimized by changing from heavy-weight “standard” pore meshes to low-weight “mega” pore meshes, which might reduce the fibrotic scar tissue formation and shrinkage around the mesh (Klinge et al., 2012). Polypropylene mesh exhibits mega pores, good tensile strength, adequate inflammatory response, and low fibrosis, and is being widely used (Van’t Riet et al., 2003; Butler et al., 2004).

Retromuscular position seems to be the most commonly used and safe mesh placement site, and the mesh must be ideally separated from abdominal organs in order to avoid adhesion formation (Montgomery, 2013). However, in large abdominal wall defects, it is very difficult to avoid contact between abdominal organs and the mesh, leading to an increased number of adhesions and other complications such as fistula formation and intestinal obstruction (Dinsmore et al., 2000; Alimoglu et al., 2003).

Polypropylene mesh can induce dense abdominal adhesions to peritoneal structures; however, adhesion avoidance is desirable. Synthetic meshes are usually designed to be positioned outside the abdominal cavity, unless a special coating has been applied for intra-abdominal use (Montgomery, 2013). Several substances were tested, but there is not yet an ideal mesh coating that would show no adverse reactions, complications, or foreign body reaction that would be simple to use, easily accessible, and have a low cost (Toosie et al., 2000; Lontra et al., 2010; Zong et al., 2004).

In the Amazon rainforest region, Andiroba oil is an herbal extract widely used by the local population to treat several inflammatory diseases. Today, the level of public policies of the World Health Organization encourages the use of medicinal plants. Because Andiroba oil has proven to have anti-inflammatory and healing effects (Brito et al., 2001; Rodrigues et al., 2008; Brito et al., 2006), it could be of great use as a mesh coating or therapeutic drug for VHR. Thus, Andiroba oil was tested as a mesh coating or by oral administration, in a VHR model of abdominal wall defect, to improve abdominal wall repair.

**MATERIALS AND METHODS**

**Animals**

Thirty-six male Wistar rats (15 to 20 weeks), weighting 250 to 300 g, were used in this study. The animals were kept in a vivarium of the Laboratory for Experimental Surgery at Para State University (Brazil) in a temperature-, light-, humidity-, and noise-controlled environment. Water and food were provided ad libitum. This research strictly followed the rules of the Brazilian National Law for Animal Care (Law: 11.794/08) that is based on NIH guidelines, and followed the rules of Council for International Organization of Medical Sciences ethical code for animal experimentation. The project was previously approved by the animal use and care committee at Para State University (AUCC-UEPA).

**Experimental protocol**

The animals were randomly assigned into the following three groups (n=12 for each group): (1) In the control group (CONT), VHR was done using only polypropylene/poliglecaprone meshes. (2) In the Andiroba oil gavage group (AndO), animals were treated with meshes and 0.63 ml/kg Andiroba oil by oral administration, 7 days prior to the mesh placement. (3) In the Andiroba oil submersion group (AndS), animals were treated with meshes that were previously submersed in Andiroba oil for 15 min immediately prior to their placement.

**Surgical procedures**

All procedures were performed in anesthetia (ketamine hydrochloride and xylazine hydrochloride 60 and 6 mg/kg, respectively, i.p.).

**Crude Carapa guianensis oleoresin oil** was obtained from the Brazilian National Laboratory for Research in Medicinal Plants. In the AndG group, oral administration was given by gavage directly into the stomach, once in a day, for 7 consecutive days prior to the surgery. The administered daily dose was 0.63 ml/kg. Such dose was previously used by Rodrigues et al. (2008), who analyzed different doses of Andiroba oil and showed that at 0.63 ml/kg there was systemic inflammatory response modulation after a kidney injury and no toxicity to other organs.

In all animals, laparotomy was performed using a 4-cm skin incision at the midline and the exposure of the aponeurotic muscle layer. Immediately after, an excision was done on the ventral part of the abdomen, involving the aponeurotic muscle layer and the peritoneum, with a 2-cm longitudinal axis and 2-cm transversal axis, in order to create a ventral defect in the aponeurotic muscle.

Ventral hernia was repaired in all groups by the placement of polypropylene/poliglecaprone meshes having 3-cm longitudinal and transversal axes, attached at the edges with eight separated stitches (6-0 nylon thread), equidistant, performing five semi-knots in each stitch and leaving the mesh margins over the anterior aponeurotic plane (Figure 1).

Animals were euthanized by lethal anesthetic doses at three time points (4 animals each group): 7, 14, and 21 days. Meshes were found through the incision that was done previously. Then macroscopic analysis was performed regarding the presence of incisional hernias, infections, dehiscence or fistulas and the total number of adhesions. After this, scar tissues above and nearby meshes and the entire mesh were harvested for histological analysis.
Histological examination

Harvested tissue was fixed in 10% buffered formaldehyde solution, embedded in paraffin, and stained using hematoxyline/eosin and Masson's trichrome.

At the hematoxyline/eosin stain, inflammatory response parameters were analyzed. Macrophage, lymphocyte, and giant cell counting around mesh fragments were performed and classified as 0=absence, 1=mild, 2=moderate, and 3=intense. Necrosis, fibrosis, and type of granuloma were classified as 0=absence, 1=mild, 2=moderate, and 3=intense. At Masson's trichrome stain, collagen fibers were quantified and classified as 0=absence, 1=mild, 2=moderate, and 3=intense.

Statistics

Normal distribution of data was confirmed using the Kolmogorov-Smirnov test. Results were analyzed by Kruskal-Wallis test. Statistical significance was assumed at p < 0.05.

RESULTS

Macroscopic analysis

Incisional hernias, infections signs, dehiscence, or fistula formation were not detected in any animal. Abdominal adhesions between abdominal organs and the mesh were detected in all animals; however, AndG animals showed fewer adhesions than CONT at the 14th and 21st days (Table 1).

Inflammatory response

All animals showed acute inflammatory response that was characterized by swelling, vascular congestion, and neutrophil infiltration. Regarding other immunologic cell counting, AndS showed a higher number of macrophages than AndG in all three time points, whereas CONT showed higher giant cells and lymphocytes counting than AndS and AndG in all three time points (Table 2).

Granulomas formed around the meshes were histologically characterized as a foreign body type response granuloma, and were composed of macrophages and giant cells around each mesh fragment observed, showing no difference on the intensity among groups.

Necrosis was observed in all animals; however, AndG showed less necrotic areas than CONT and AndS in all three time points. Fibrotic areas appeared only at the 21st day on CONT, but have been identified since the 7th day on AndG and AndS. AndS showed higher degrees of fibrosis in all three time points and AndG showed a fibrosis degree similar to CONT at the 21st day (Table 3).

Collagen synthesis

CONT and AndG showed increased collagen fibers within postoperative days; however, AndS showed a rapid increase during the first postoperative week and subsequent stabilization of collagen fibers in growth. AndG showed a faster ingrowth of collagen fibers than CONT, reaching its peak on Day 14 (Figure 2).

DISCUSSION

After abdominal wall surgeries, postoperative pain, which is a major patient complaint, can be diminished using non steroidal anti-inflammatory drugs; however, these drugs can slow down the healing process and increase the chances of complications (Alimoglu et al., 2003). Some anti-inflammatory drugs have already been tested on VHR, and the results remain controversial. Andiroba oil shows anti-inflammatory, wound healing, and antimicrobial actions, thus it could be of great application for a better VHR outcome as it could modulate systemic inflammatory response, avoid infections, and lead to a better wound healing outcome (Brito et al., 2001; Rodrigues et al., 2008; Brito et al., 2006; Santos et al., 2012).

The absence of dehiscence, fistula formation, or infections in all animals demonstrates that Andiroba oil did not strongly interfere in the early abdominal wall healing process; early changes occurred in studies using parecoxibe (Kyriakidis et al., 2011) and meloxicam (Tognini, et al., 2000). Macroscopic results were similar to those found by Pundek et al. (2010), who analyzed that VHR was analyzed when only polypropylene/polyglycaprone meshes were used.
Figure 2. Collagen fibers within days. P < 0.05 AndG vs. CONT at 14th day; AndG vs. AndS at 21st day.

Table 1. Average number of adhesions between the mesh and abdominal organs.

<table>
<thead>
<tr>
<th>Group</th>
<th>CONT</th>
<th>AndG</th>
<th>AndS</th>
</tr>
</thead>
<tbody>
<tr>
<td>7th day</td>
<td>2.75</td>
<td>1.75</td>
<td>2</td>
</tr>
<tr>
<td>14th day</td>
<td>3*</td>
<td>1.5*</td>
<td>2.5</td>
</tr>
<tr>
<td>21st day</td>
<td>3.75*</td>
<td>1.5*</td>
<td>2.75</td>
</tr>
</tbody>
</table>

*Statistical difference; p < 0.05. CONT vs. AndG at 14 and 21 days (Kruskal-Wallis test).

Table 2. Groups average of inflammatory cell counting surrounding mesh fragments.

<table>
<thead>
<tr>
<th>Group</th>
<th>CONT</th>
<th>AndG</th>
<th>AndS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th day</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>14th day</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>21st day</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Giant cells#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th day</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>14th day</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>21st day</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lymphocytes#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th day</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>14th day</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>21st day</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*p < 0.05 AndG vs. CONT and AndS (Kruskal-Wallis test); *p < 0.05 CONT vs. AndG and AndS (Kruskal-Wallis test).
Abdominal adhesions can occur in up to 90 to 100% of all abdominal surgeries and can lead to severe complications, such as fistula formation and intestinal obstruction (Buerger et al., 2006). Almost all meshes may produce adhesions when they are in contact with the intestinal surface, and this process is determined by the size of the pores and the structure of the mesh (Klinge et al., 2012).

Oral administration of Andiroba oil was able to reduce the number of adhesions between the mesh and the abdominal organs when compared with CONT (Table 1). This fact probably occurred due to modulation of the inflammatory response by Andiroba oil, reducing aggression between the parietal and visceral serosa, and thereby reducing the formation of adhesions (Ricciardi et al., 2012).

When Andiroba oil was used as a mesh coating, abdominal adhesions were similar to CONT. It occurred probably, because Andiroba oil was not systemically absorbed and acted as a local chemical irritant, contributing to adhesion formation (Souza Junior et al., 1999). This fact was confirmed by higher macrophage counting on the AndS group, showing a greater foreign body type response.

A reduced number of lymphocytes and giant cells were the reason for less local inflammatory response in both Andiroba groups (Table 2) as the lymphocytes are responsible for inflammatory response augmentation (Ferrante et al., 2012).

Necrotic areas occurred due to foreign body type inflammatory response. All meshes will introduce a foreign body type response that needs to be balanced in order to result in normal wound healing. Chemicals can remain within the mesh, causing both toxic and inflammatory reactions in the patient (Montgomery, 2013). AndG was the only group that showed reduced necrosis areas in all three time points analyzed, confirming that oral administration of Andiroba oil can modulate the inflammatory response secondary to VHR.

During VHR, there are many steps that can fail; early degradation or absorption of the mesh and fibrosis formation are undesirable (Montgomery, 2013). Andiroba oil inflammatory response modulation did not significantly interfere with fibrosis formation, which was similar in both AndG and CONT groups (Table 3). Albeit, when the herbal extract was used as a mesh coating, there were more intense fibrosis and mesh reabsorption, probably due to the greater foreign body type response (Ferrante et al., 2012).

When collagen fiber ingrowth was analyzed (Figure 2), AndG and CONT groups had the same final amount of collagen fibers deposited on the mesh; however, oral administration of Andiroba oil accelerated collagen fiber formation, reaching its peak one week before the control group. On the other hand, when Andiroba oil as a mesh coating was tested, there was less collagen fibers ingrowth when compared with the other groups, exposing that there was worse tissue healing.

There is unceasing search for an ideal mesh coating that would show no adverse reactions, complications, or foreign body reaction, and one that is simple to use, easily accessible, and affordable (Montgomery, 2013). However, this study shows that no substance, despite its anti-inflammatory or tissue healing activity, should be used in the clinical setting of VRH before a full basic research is done, because Andiroba oil used as a mesh coating showed greater fibrosis and less collagen fiber formation, leading to worse VHR outcome.

On the other hand, oral administration of Andiroba oil showed promising results on this adopted experimental setting of VHR. It was able to modulate the systemic inflammatory response, reducing abdominal adhesion formation; interestingly, it did not diminish tissue healing, had low ratio of fibrosis, and had a faster and satisfactory collagen fiber formation. This fact shows that there is a wide field of study for the development of new medicinal plant-related drugs that could be used in the clinical setting of VHR.

Conclusion

Oral administration of Andiroba oil modulated inflammatory response, reducing abdominal adhesion formation; interestingly, it did not diminish tissue healing, had low ratio of fibrosis, and had a faster and satisfactory collagen fiber formation, delivering a better VHR outcome. However, when it was used as a mesh coating, greater fibrosis and less collagen fibers formation were seen, exposing worse VHR outcome.

**ABBREVIATIONS**

VHR, Ventral hernia repair; AndG, Andiroba oil gavage group; AndS, Andiroba oil submersion group; CONT, control group.

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**Table 3.** Average of necrosis and fibrosis area among groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>CONT</th>
<th>7th day</th>
<th>14th day</th>
<th>21th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis</td>
<td>Fibrosis</td>
<td>Necrosis</td>
<td>Fibrosis</td>
<td>Necrosis</td>
</tr>
<tr>
<td>AndG</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1.75</td>
</tr>
<tr>
<td>AndS</td>
<td>2.25</td>
<td>0.75</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*p < 0.05 AndG vs. CONT (Kruskal-Wallis test); *p < 0.05 AndS vs. AndO (Kruskal-Wallis test).
Conflict of Interest

Authors declare no conflict of interest.

REFERENCES


REFERENCES


