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Effect of *Sargassum sp.* extract on preventing abdominal adhesions in female rat

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Abstract

Background: Studies have shown that abdominal adhesions occur due to an oxidative stress by ROS. *Sargassum sp.* extract contains many antioxidative compounds that might be utilized to interrupt this process. This study intends to study the efficacy of *Sargassum sp.* extract on preventing abdominal adhesions.
Methods: In this study, 20 female rats were divided into two groups. Group 1 receive *Sargassum sp.* extract and group 2 served as controls. Intra-peritoneal ketamine was administered for anesthesia. Multiple scratches were made in the peritoneum and then, the abdomen was closed. After two weeks, a sample of the peritoneum was sent to the laboratory for adhesion grading and histo-pathological examination. Data were analyzed by IBM SPSS.
Results: There was significant difference in adhesion found between the two groups ($p<0.05$). There was also a significant difference regarding vascular proliferation between two groups.
Conclusion: Present study shows that applying *Sargassum sp.* extract can tremendously reduce post operation abdominal adhesions.

Keywords: Abdominal Adhesions; *Sargassum sp.*; Female Rat

Introduction

Intra-abdominal adhesions are fibrous bands of peritoneal tissue that make intra-abdominal organs join each other or to the abdominal wall. These adhesions are known as a complication in healing following surgery or infection and can lead to serious consequences [1, 2]. Studies revealed that approximately 93% of patients develop intra-abdominal adhesions after abdominal surgery [3]. Because of importance of the health problems and financial consequences related to the adhesions, prevention or reduction of postoperative adhesions have been an important priority of many articles but several controversies still exist [4]. Adhesions can form when the parietal or visceral peritoneum is damaged and the basal membrane of the mesothelial layer is exposed to the surrounding tissues, resulting in tissue ischemia, inflammation, fibrin deposition, fibrin organization, collagen formation, and maturation with the formation of adhesions [1, 2, 5, 6].
Adhesion mechanism is known as a continuous infla mmatory event due to the presence of ROS (Reactive oxygen species) [7]. ROS might cause a damage because they can initiate biomolecular oxidations which lead to cell injury and death. There are evidence that indicate ROS are responsible for many diseases and biological damages [8-10]. Multiple previous documents have shown that the activity of ROS could be reduced by using antioxidants [11]. While marine algae as expected are exposed to a combination of light and oxygen which results in the formation of free radicals and other strong oxidizing agents, there is a reported absence of oxidative damage in their structural components [11-15]. *Sargassum sp.* has been investigated for several possibilities [13]. *Sargassum sp.* is the name of the

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algae proliferating in tropical and subtropical water blocks around the world, growing along beaches with a rocky substrate, rolling stones and pebbles [13, 16]. It has been shown that *Sargassum sp.* extract contains antioxidants [16-18]. Previous studies illustrated the beneficial compounds of *Sargassum sp.* extract as capable ofinhibiting inflammatory productions. The phenolic compounds were considered as one of the most effective antioxidant in brown algae [19]. In general, many surveys have shown that phlorotannins were the main phenolic compounds detected in brown algae [20, 21]. Phlorotannin is a group of phenolic compounds that are formed by the polymerization of phloroglucinol (1, 3, 5 trihydroxybenzene) monomer units and synthesized in the acetate-malonate pathway in marine algae [22, 23]. Inflammatory process occurs in response to trauma, infection, tissue injury or noxious stimuli [24, 25]. In this process, activated inflammatory cells secrete increased amounts of nitric oxide (NO), prostaglandin E2 (PGE2) and cytokines, such as interleukin (IL)-1ß, IL-6, and tumor necrosis factor (TNF). These substances induce cell and tissue damage [26-29]. Most importantly, iNOS is highly expressed in macrophages; its activation leads to organ destruction in some inflammatory states and also is associated with adhesions. There isevidence that *Sargassum sp.* extract can strongly inhibit NO productions and the subsequent inflammation [30]. Therefore the present study aimed to show the effects of *Sargassum sp.* extract on preventing intra-abdominal adhesions in female rats.

Methods

Twenty female Wistar rats (weighing 200-250g) were purchased from Hormozgan Medical University animal unit and were housed in environmentally controlled conditions (22±20 C, 12- hour light-dark cycle), with free access to standard pellet diet and water. They were divided to two groups of 10: group 1 being the case and, group 2 being the control.

Surgery and adhesion induction

The animals were kept NPO for 12 hours before surgery. The anesthesia performed with an ip injection of ketamine (30mg/Kg). The surgical procedures were performed under sterile conditions. After hair removal, the abdomen was cleaned with 1% antiseptic povidone-iodine solution and a 3-cm midline laparotomy was made, the peritoneal wall was completely removed using sharp forceps. All the surgeries were carried out by the same person under one approximately similar condition. The animals' nutrition was controlled for two weeks. There were two animal losses in the control group that were replaced. No animal in experimental group was dead or replaced.

Solutions, Extraction and Injection

The applied solution containing extract of *Sargassum sp*. made in Marine Biology Department of Hormozgan University. Two hot-water extract procedures were carried out and the solution was then filtered through antiseptic filter in order to earn sterile solution. Each injection contained 200 mg of the extract. The control group received no injection (or) received deionized water as vehicle

Adhesion Assessment

Two weeks after the surgery, the animals were sacrificed, and the abdominal cavity was shaved and opened via an 8-cm incision based in the lower abdomen for complete exploration. Blind evaluations were carried out; adhesions were graded from 0 (absent) to 4 (severe) according to the Mazuji classification (Table 1) [31]. In general, Grades 0 and 1 adhesions have no clinical significance, whereas Grades 3 and 4 adhesions can be a cause of intestinal obstruction. Adhesion rate is used to describe the presence of an adhesion of any grade. After biopsy; the samples were fixed in formalin and sent to pathology lab for histology evaluations. The findings were compared with Adhesion Grading Classification.

Table 1. Adhesion grading according to Mazuji classification

Grade	Description of Grade
0	No adhesion
1	Very small, irregular adhesion
2	Easily separable medium intensity adhesion
3	Intense, not easily separable regular adhesion
4	Very intense, not easily separable, homogeneous adhesion

Histology

Histopathologic examination was performed by two investigators. Adhesion-carrying tissues were excised en- block and fixed in a 10% buffered formaldehyde solution. Histological study was performed after

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staining with hematoxylin and eosin. The evaluated parameters were fibrosis, inflammation, and vascular proliferation, rated on a modified semi-quantitative scale of 0-3 [32-34]. The amount of fibrosis was scored as follows: 0, no fibrosis; 1, minimal, loose fibrosis; 2, moderate fibrosis; and 3, florid dense fibrosis. Inflammation was scored as follows: 0: no inflammation; 1: presence of giant cell, occasional lymphocytes and plasma cell; 2: presence of giant cell, plasma cells, eosinophils and neutrophils; and 3: presence of many inflammatory cells and micro-abscesses. Vascular proliferation was scored as: 0: no vascular proliferation; 1: mild vascular proliferation; 2: moderate vascular proliferation; and 3: intense vascular proliferation.

Statistical Analysis

Statistical analysis was performed using the IBM SPSSversion 17. Differences in the numbers of animals without adhesions in the different treatment groups were evaluated using a Chi-square test. Independent two-tail t-test was used to determine differences between two independent populations.Kruskal-Wallis and Mann- Whitney U-test were used whenrequired. A p-value≤ 0.05 was considered statistically significant.

Results

Data analysis showed grade 3 of adhesion in % 40 of group 2 rats. There was also no rat without adhesion in this group (table 2). Whereas merely 30% of group 1 showed grade 1, once grade 2 and then 70% reported negative. Therefore significant differences of adhesion inducing, found between two groups (p <0.05).

Table 2. Adhesions grades and histological Histologic features in control group

No.	Adhesion Grade	Fibrosis	Inflammation	Vascular Proliferation
1	3	3	1	1
2	1	2	1	2
3	2	3	2	3
4	2	3	3	0
5	3	3	1	1
6	2	2	3	0
7	3	3	2	2
8	4	2	3	3
9	3	3	3	2
10	4	1	1	0

Results of histological investigations were related not only positive in fibrosis and inflammation among group 2 but also negative among group 1 ($p<0.05$). There were also significant relationship found, in vascular proliferation between two groups. It was indicated that 90% of group 1 had no vascular proliferation, though 70% of group 2 stated positive (table3).

In general severity of adhesion as well as positive results of the histological investigations were mentioned much higher in group two in comparison with group two which received extract of *Sargassum sp*. In these amongst, the most common (in the control group) adhesion severity was intense (grade 3), dense fibrosis, micro-abscesses and moderate vascular proliferation.

Table 3. Adhesions grades and histological Histologic features in experiment group

No.	Adhesion Grade	Fibrosis	Inflammation	Vascular Proliferation
1	0	0	0	0
2	1	0	1	0
3	0	0	0	0
4	2	1	1	1
5	0	0	2	0
6	1	0	2	0
7	1	1	1	0
8	0	0	0	0
9	0	0	1	0
10	0	0	0	0

Discussion

The process of post operation adhesions occurs due to several suggested mechanisms [4]. Schnüriger. B, et al, accused inflammation as the cause [4]. In many studies ROS considered the background factor in triggering inflammation [35-38]. Other studies suggested, using antioxidants in order to control ROS and then inflammation [7, 35, 39]. Previous researches on adhesion prevention, applied different materials such antibiotics and antioxidants [40, 41]. Aram, S., et al, tried vitamin E, and Woollard, K., et al,used vitamin C, both hoped to reduce the adhesion which were partially attained. Similar to Durmus, A. S., et al, which used selenium, Özçelik, B., et al also applied melatonin, they supposed that antioxidative activities of these productions reduced post operation adhesions. Very few studies have

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been carried out to show efficacy of natural antoxidative plants or algae on preventing post operation adhesions [36]. As mentioned before, in the present study, it was hypothesized that, due to proved antioxidative activity of *Sargassum sp.* extract, it is likely to have efficacy on post operation adhesions. Probable mechanism is that, NO is responsible for developing inflammation, can be extremly inhibited by *Sarggasum sp* extract. As it has been reported in prior studies, it includes Total Phenolic Content (TPC) which is likely to make it capable for inhibiting inflammatory process [35].

According to the findings, especially histological results, it seems that the hypothesis tremendously been proved and then applying it could reduce post operation adhesions. There was no evidence that *Sarggasum sp* extract be containing any cell toxicities productions. Using other materials include acid components such as vitamin C can probably trigger to inflammation and vascular injuries. Vitamin E and other hydrophobic materials would not removed as soon as hydrophilic materials, and other chemical materials, to have synthetics components, would postpone healing process. In these amongst, *Sarggasum sp* can be applied as hydrophilic and its acidity can be control perfectly [15].

However previous study stated that *Sarggasum sp* in 200 mg dosage has convincing antioxidative activity. This dose also applied in the present study, but it seems that more study is required to determine the precise mechanisms of action as well as a better dose-response relation.

Conclusions

This study evaluated the effect of *Sarggasum sp extract* on preventing post-operative adhesions. During the research it has been observed that application of mentioned this extract can tremendously inhibit adhesions process.

Conflict of Interest

The authors declare that they have no conflict of interests.

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