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Experimental endometriosis reduction in rats treated with *Uncaria tomentosa* (cat's claw) extract

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ABSTRACT

Objective: The aim of this study was to analyze the macroscopic and histological changes that occur in experimental endometriosis after treatment with *Uncaria tomentosa*.
Study design: Experimental endometriosis was induced in twenty-five female Wistar rats. After three weeks, 24 animals developed grade III experimental endometriosis and were divided into two groups. Group "U" received *U. tomentosa* extract orally (32 mg/day), and group "C" (control group) received a 0.9% sodium chloride solution orally (1 ml/100 g of body weight/day). Both groups were treated with gavage for 14 days. At the surgical intervention and after the animal was euthanized, the implant volume was calculated with the following formula: $[4\pi\ (\text{length}/2) \times (\text{width}/2) \times (\text{height}/2)/3]$. The autotransplants were removed, dyed with hematoxylin–eosin, and analyzed by light microscopy. The Mann–Whitney test was used for the independent samples, and the Wilcoxon test analyzed the related samples, with a significance level of 5%.
Results: The difference between the initial average volumes of the autotransplants was not significant between the groups ($p = 0.18$). However, the final average volumes were significantly different between the groups ($p = 0.001$). There was a significant increase ($p = 0.01$) between the initial and final average volumes in the control group, and treatment with the *U. tomentosa* caused a marked reduction in the growth over time ($p = 0.009$). Histologically, in the experimental group ($n = 10$) six rats had a well-preserved epithelial layer, three had mildly preserved epithelium, and one had poorly preserved epithelium. The epithelial layer occasionally presented sporadic epithelial cells. The control group ($n = 12$) presented seven cases (58.3%) of well-preserved epithelial cells and five cases (41.7%) of mildly preserved epithelial cells.
Conclusions: Cat's claw extract appears to be a promising alternative for treating endometriosis.

1. Introduction

Endometriosis management may be clinical, based on induction of hypoestrogenism, or surgical, including lesion excision and pelvic anatomy repair. The medications available today to treat endometriosis act by blocking estrogen secretion from the ovaries, which leads to implant regression and remission of symptoms. However, this type of treatment fails to yield any healing effect, as symptoms usually resurface after suspension of the medication [1]. Surgical treatments are also usually unable to heal the patient completely, and these types of intervention are not free of recidivism or complications. These realities are the rationale for the search for novel treatment approaches. Due to improved knowledge on the etiopathology of the disease, there have been large efforts in several experimental models to find drugs with mechanisms of action that interfere with the steps already known to be involved in disease initiation and progression [2–5].

Several cellular and molecular changes have been described for both endometrial implants and the affected peritoneal setting. Ectopic implants are estrogen and progesterone responsive, producing a series of immunomodulators, inflammatory mediators and proteins involved in the oxidative process at the end stages of the menstrual cycle [6–8]. Pelvic inflammation reactions in patients with endometriosis are related to local and systemic immunological manifestations through the buildup of inflammatory cells and the increased production of inflammatory cytokines such as interleukin 1, TNF-α, interleukin 6 and interleukin 8 [9].

Uncaria tomentosa, an herb from the *Rubiaceae* family, popularly known as "cat's claw" in Brazil, is native to Tropical Central and South America. In several parts of the world it is used for treating infections with inflammatory or oxidative stress. The plant has

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anti-inflammatory properties mediated by the inhibition of lipopolysaccharides, nitrites and prostaglandin E_2 (PGE_2). Additionally, it can alter cell cycle progression by inducing apoptosis and functions in an anti-oxidizing capacity acting as a free radical eliminator [10–14]. Treatment with *U. tomentosa* inhibits the production of TNF-α, a powerful pro-inflammatory cytokine and critical mediator of chronic inflammatory states. TNF-α inhibition is controlled by the regulation of the NFκB transcription factor, which may regulate the expression of several pro-inflammatory cytokines including TNF-α, IL-1, IL-2, IL-6 and IL-8 [11].

The development of experimental endometriosis in female rats, through auto-transplantation techniques, prompted research on additional medical treatments for endometriosis. These treatments are analyzed for their efficacy via macroscopic and histological parameters, among others [15,16]. Because of the known properties of *U. tomentosa* and its relationship to mechanisms involved in the etiopathogeny of endometriosis, the present study aimed to analyze its effects on experimental endometriosis implants in rats.

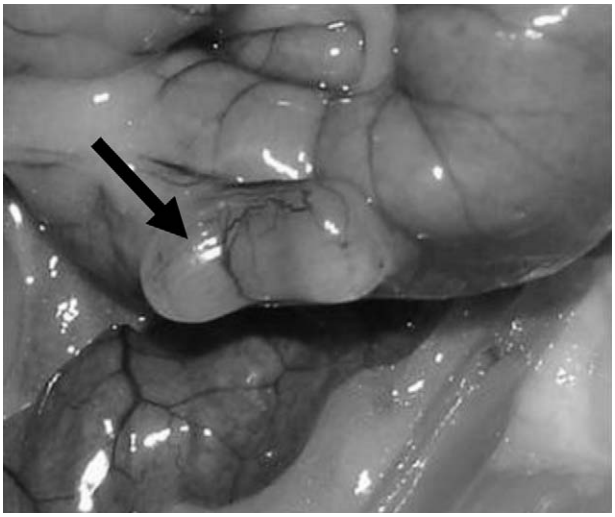

Fig. 1. Photomicrography showing an autotransplant with a diameter longer than 4.5 mm, classified as grade III according to Quereda et al.

2. Materials and methods

The study was carried out between July and September 2009, using 25, 60-day-old Wistar (*Rattus norvegicus albinus*) female, adult rats weighing 180–250 g. The rats were obtained from the Federal University of Maranhão (UFMA) Bioterium.

The study was developed in the Experimental Surgery Laboratory of the University Hospital of the Federal University of Maranhão, Brazil. Study procedures observed regulations from the Brazilian Legislation for the use of experimental animals (Arouca Act no. 11.794/2008) and from the Colégio Brasileiro de Experimentação Animal (COBEA), an institution affiliated with the International Council for Laboratory Animal Science. The study was approved by the Ethics Committee and Animal Experimentation (CEEA-UEMA) under protocol number 004/09.

The animals were grouped four per polypropylene cage (46 cm × 31 cm × 16 cm) with a stainless steel grid lid and floorboards covered with paper that was replaced every 48 h. The animals were divided into four groups and maintained under constant environmental conditions, including rat rations (PURINA®, São Paulo, Brazil) and water *ad libitum*, for seven days for adaptation, noise control, 22 ± 2 °C temperature, 40–60% relative humidity and 12/12 h light/dark cycles.

The autotransplantation technique was performed according to the methodology proposed by Nogueira et al. [3]. Shortly after the midway incision, the uterine horns were identified; fragments of the medium third were resected, immersed in saline solution and cut into 4 mm × 4 mm fragments. The fragments were sutured to the mesentery adjacent to the artery that irrigates the cecum, with the serosal surfaces turned to the peritoneum and the endometrial layer turned to the cavity.

After the first surgery, the animals were kept in the laboratory for a period of 21 days. After this period, the rats underwent an additional operation; an inventory of the peritoneal cavity was taken using a digital pachymeter to identify the success of the autotransplantation, followed by a volume calculation using this formula: $[4\pi\ (\text{lenght}/2) \times (\text{width}/2) \times (\text{height}/2)/3]$ [17]. Classification of the experimental endometriosis implant growth was performed according to Quereda et al. [15], and only those animals that progressed to a growth score III remained in the study (Fig. 1).

After the surgical approach, the rats were identified and randomly divided into *U. tomentosa* (U Group) and control groups (C group), both containing 12 rats.

Gavage of *U. tomentosa* extract (UNHA DE GATO® 100 mg, Herbarium do Brazil) at 32 mg/day for 14 days was carried out for the U group according to the modified instructions by Moreno et al. [18]. The C group received 1 ml daily gavage of 0.9% saline solution for 14 days.

After the end of the medical treatments, the rats were euthanized using ketamine anesthesia, and a third laparotomy was performed. After opening the abdominal wall, an inventory of the peritoneal cavity and measurements of autotransplant volumes were performed; the transplant and the middle third of the remaining uterine horn were then removed. The salvaged tissue was rinsed with 0.9% saline solution and stored in 10% formaldehyde buffer for later anatomopathological analysis.

Paraffin tissues were sectioned in 5-μm widths and placed in a warm bath; the slides were incubated with Meyer albumin and dried afterwards. Tissue sections were stained with hematoxylin–eosin (HE), and histological analysis was performed by one pathologist. The estrous cycles were analyzed in the middle third of the uterine horn remaining and drug efficacy was evaluated according to the criteria explained by Keenan et al. [16]. The persistence of epithelial cells in uterine autografts was evaluated as follows: a well-preserved epithelial layer = score 3, a moderately preserved epithelium with leukocyte infiltrate = score 2, a poorly preserved epithelium (occasional epithelial cell only) = score 1, and no epithelium = score 0.

Biostat 3.0 Windows XP was used for statistical analysis, where the significance level (α) used to reject the null hypothesis was 5% ($p < 0.05$). The Mann–Whitney test was used for independent samplings, and the Wicoxon test was performed for related samples.

3. Results

There were two broncho-aspiration deaths in the experimental group, which left ten rats, compared with twelve rats in the control group. All the middle third of the remaining uterine horn was in the proestrus or estrus phase of estrous cycle. There was no significant difference between the mean volume (38.10 mm^3) in the control group and (45.53 mm^3) and that in the U group at 21 days after induction of endometriosis ($p = 0.18$). Two weeks after administering cat's claw and the saline solution, the final average volumes were 75.70 mm^3 for the control group and 27.26 mm^3 for the cat's claw group ($p = 0.001$).

Average volumes of the initial control group and the saline-treated group two weeks later were 38.10 mm^3 and 75.70 mm^3, respectively. This increase in average volume was significantly different ($p = 0.01$). For the cat's claw group, the initial average