



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.

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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 E₂ (PGE₂). 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 NF κ B 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.

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 \times 31 cm \times 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 \pm 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 \times 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: $[4\pi (\text{length}/2) \times (\text{width}/2) \times (\text{height}/2)/3]$ [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.



Fig. 1. Photomicrography showing an autotransplant with a diameter longer than 4.5 mm, classified as grade III according to Quereda 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- μ m 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 mm³) in the control group and (45.53 mm³) 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 mm³ for the control group and 27.26 mm³ 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 mm³ and 75.70 mm³, respectively. This increase in average volume was significantly different ($p = 0.01$). For the cat's claw group, the initial average

Table 1

Characteristics and results for a group of rats treated orally for 14 days with cat's claw (32 mg/day) or saline solution (1 ml/100 g body weight).

Group	Average initial volume	Average final volume	p^*
Cat's claw	45.53 mm ³	27.26 mm ³	0.009
Control	38.10 mm ³	75.70 mm ³	0.010
p^{**}	0.187	0.001	

* The Wilcoxon test for samples (with $p < 0.05$ for rejecting the null hypothesis) comparing averages of the initial and final volumes between the groups.

** Mann-Whitney test for independent samples (with $p < 0.05$ for rejecting the null hypothesis) comparing averages of the initial and final volumes between the cat's claw and the control treated groups.

volume was 45.53 mm³, in contrast to the 27.26 mm³ final average, and this difference was statistically significant ($p = 0.009$) (Table 1).

Regarding preservation of the epithelial layer, upon histological evaluation of the autotransplant focus, the experimental group ($n = 10$) contained six (60%) rats with a well-preserved epithelial layer (Fig. 2), three cases (30%) with mildly preserved epithelium, and one (10%) with poorly preserved epithelium. The epithelial layer occasionally presented sporadic epithelial cells (Fig. 3). The control group ($n = 12$) included seven cases (58.3%) with well-preserved epithelial cells and five cases (41.7%) of mildly preserved epithelial cells.

4. Comments

Endometriosis is a chronic, debilitating illness that leads to a very poor quality of life. The surgical treatment, usually laparoscopic, is based on the removal of all lesions, which at times brings about mutilating surgeries with short-, medium-, and long-term sequelae. Based on these issues, therapeutic approaches have been used more frequently in the management of this enigmatic disorder. Today, medical therapy for endometriosis consists of medications that generally interfere with the ovarian hormonal cycle [1]. Such medications, normally used for an extended period of time, generate unwanted side effects and

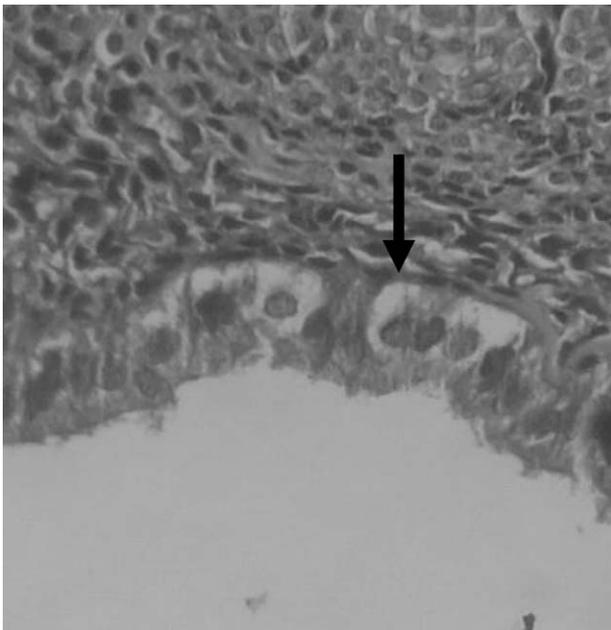


Fig. 2. Photomicrography of well-preserved epithelial cells, with well-preserved (40 \times) cytoplasm from the group of rats treated with 32 mg/day of *Uncaria tomentosa*, administered for a 14-day period.

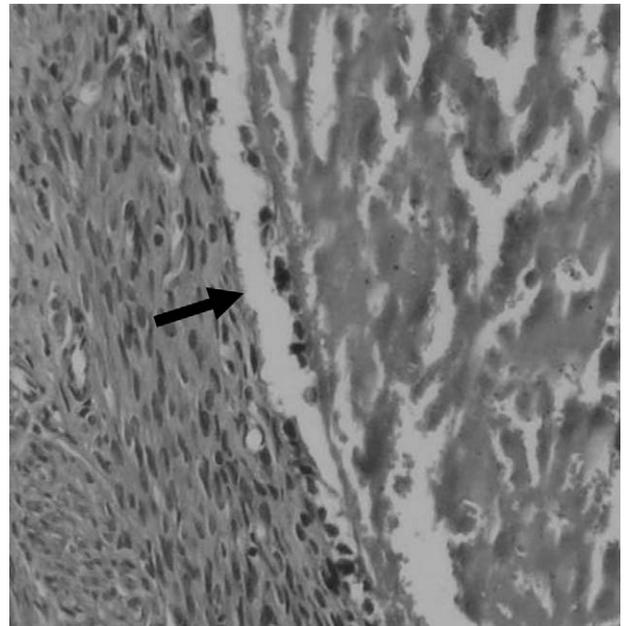


Fig. 3. Photomicrography of well-preserved epithelial cells, with mildly preserved (40 \times) cytoplasm from the group of rats treated with 32 mg/day *Uncaria tomentosa*, administered for a 14-day period.

should be taken with caution by women who smoke or who have had prior thromboembolic incidents. A better understanding of endometriosis pathology has prompted an interest in searching for drugs capable of interfering with the development and maintenance of the disease. Specific drug effects of interest include inhibition of cell growth, anti-inflammation, immunomodulation, or anti-oxidant properties [1–8,19].

Experimental rat models are widely used to study the effects of new drugs that have not been evaluated in humans. Several studies have analyzed the effects of drugs like aromatase inhibitors, gestrinone, analogous agonists and GnRH antagonists. Therefore, we chose an experimental rat model to examine the effect of cat's claw. This medication has been previously evaluated in treating several illnesses that have cell proliferation, inflammation, immunomodulation, or oxidative mechanisms in their physiopathology [17,20,21].

The inflammatory process of endometriosis is related to local and systemic immunological manifestations resulting from the buildup of inflammatory cells and the increased production of inflammatory cytokines [9], which validate the use of cat's claw in treating this disorder. The reduction of endometrial implants associated with decreased inflammatory processes in rat endometriotic lesions treated with dexamethasone indicates the effectiveness of such drugs in treating endometriosis [22]. The anti-inflammatory action of *U. tomentosa* has been previously proven [12], and in our research, a significant reduction of autotransplants may be due to the drug acting on the inflammatory process.

In addition to the drugs with anti-inflammatory effects, the immunomodulators showed promising effects in treating experimental endometriosis. The use of the levamisole proved effective in reducing implants of ectopic endometrium in experimental endometriosis [4]. Ciglitazone, another immunomodulator, showed a significant decrease in the number of ectopic foci in a rat model of endometriosis, even though histologically folliculogenesis and ectopic endometrial architecture remained intact [23]. These results help confirm, in practice, that the immunomodulator roles of a drug contribute to the treatment of endometriosis. These findings agree with those observed in our research involving the use of *U. tomentosa*, which also has an immunomodulator effect, as

demonstrated by the significant reduction of endometrial foci in the current study [24].

It is worth mentioning also that elevated levels of autoantibody markers for oxidative stress have been found in the pelvis of patients with peritoneal endometriosis [10]. Cat's claw is an antioxidant affecting mechanisms of cell death through the elimination of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical [11,12]. Thus, the antioxidative effect can be added to the anti-inflammatory one, which leads to a significant reduction in the size of the implants in our study. Melatonin has anti-inflammatory, immunomodulator and anti-oxidative effects, and it has been previously tested on experimental endometriosis [25]. These findings demonstrate a significant decrease of implants in the experimental group compared to the control group, similar to the results from the present study using *U. tomentosa*. This strengthens the hypothesis that anti-oxidative medications are promising for treatment of endometriosis. Statins are another class of drugs already tested on experimental endometriosis, and they appear to have inflammatory effects, in addition to inhibiting cell proliferation, reducing angiogenesis and lowering oxidative stress [3]. The use of high dose (2.5 mg/kg/day) atorvastatin showed significant reductions of endometrial foci and vascular endothelial growth factors, using experimental endometriosis models in rats [5]. The use of 20 mg/kg/day simvastatin for 14 days demonstrated a significant reduction of endometrial foci in the experimental group compared to the control group [2].

The use of 32 mg/day of *U. tomentosa* extract during 14 days led to a significant decrease of the implants in experimentally induced endometriosis in rats. The use of 32 mg/day in the present study was based on research that determined the oral ingestion effect of *U. tomentosa* on the biodistribution of sodium pertechnetate in Wistar female rats for a seven-day period [18]. The 14-day treatment utilized in the current study was based on an experimental usage stage of some drugs for induced endometriosis [3,23]. In addition to evidence that changes in inflammation, immunology and oxidative stress are somehow involved in the physiopathology of endometriosis, *U. tomentosa* can act upon these diverse processes. Combined with our results, these conclusions suggest that cat's claw could be a promising alternative for treating endometriosis.

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