

桂枝茯苓丸單方抑制子宮內膜異位症之療效

The effect of each individual herb of Guizhi Fuling Wan on inhibiting endometriosis development

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ABSTRACT

**Objectives:** Endometriosis is a common gynecological problem primarily manifested by chronic pelvic pain and infertility. An increasing body of evidence demonstrates the immunological etiology of endometriosis. Increased inflammation has been demonstrated in patients with endometriosis. In traditional Chinese medicine (TCM), endometriosis is thought to be accompanied with stagnation of vital energy (qi) and circulation. Guizhi Fuling Wan (GFW) was first described in Chinese canonical medicine treating stagnation of qi and circulation. GFW consists of *Cinnamomum cassia* Presl (Guizhi), *Poria cocos* Wolf (Fuling), *Prinus persica* (L.) Batsch (Taoren), *Paeonia lactiflora* Pall (Shaoyao), and *Paeonia suffruticosa* Andrews (Moutanpi). Previous studies demonstrated inhibition of endometriosis by GFW. The efficacy of an herbal formula can be improved by adjusting the doses of various components included in it. Thus, this study aimed to further examine the relative importance of each herb of GFW in treating endometriosis.

**Methods:** Endometriosis was generated by suturing endometrial tissue on the peritoneal wall of C57BL/6NCrIBltw mice. The mice were subsequently treated with or without GFW, GFW with sequential deletion of single herb, and single herb of GFW for 28 days. The expression of intercellular adhesion molecule 1 (ICAM-1) and caspase-3 (C-3) was examined by immunohistochemistry (IHC) and Western blot (WB).

**Results:** The endometriosis development-inhibiting effect of GFW was significantly reduced by the deletion of Guizhi and Fuling. The ICAM-1 expression-reducing effect of GFW was significantly decreased by the deletion of Guizhi, Fuling, and Taoren. The C-3 expression-elevating effect of GFW was significantly reduced by the deletion of Guizhi and Fuling. The intraperitoneal macrophage population-reducing effect of GFW was significantly reduced by the deletion of Guizhi, Fuling, Taoren, and Shaoyao. The intraperitoneal B-cell population-elevating effect was significantly reduced by the deletion of Guizhi, Fuling, Taoren, and Moutanpi. On the other hand, the endometriosis development was significantly inhibited by Guizhi, Fuling or Taoren alone. The ICAM-1 expression was significantly reduced by Guizhi or Fuling alone. The C-3 expression was significantly elevated by Guizhi, Fuling or Taoren alone. The intraperitoneal macrophage population was significantly reduced by Guizhi, Fuling, Taoren or Moutanpi treatment.

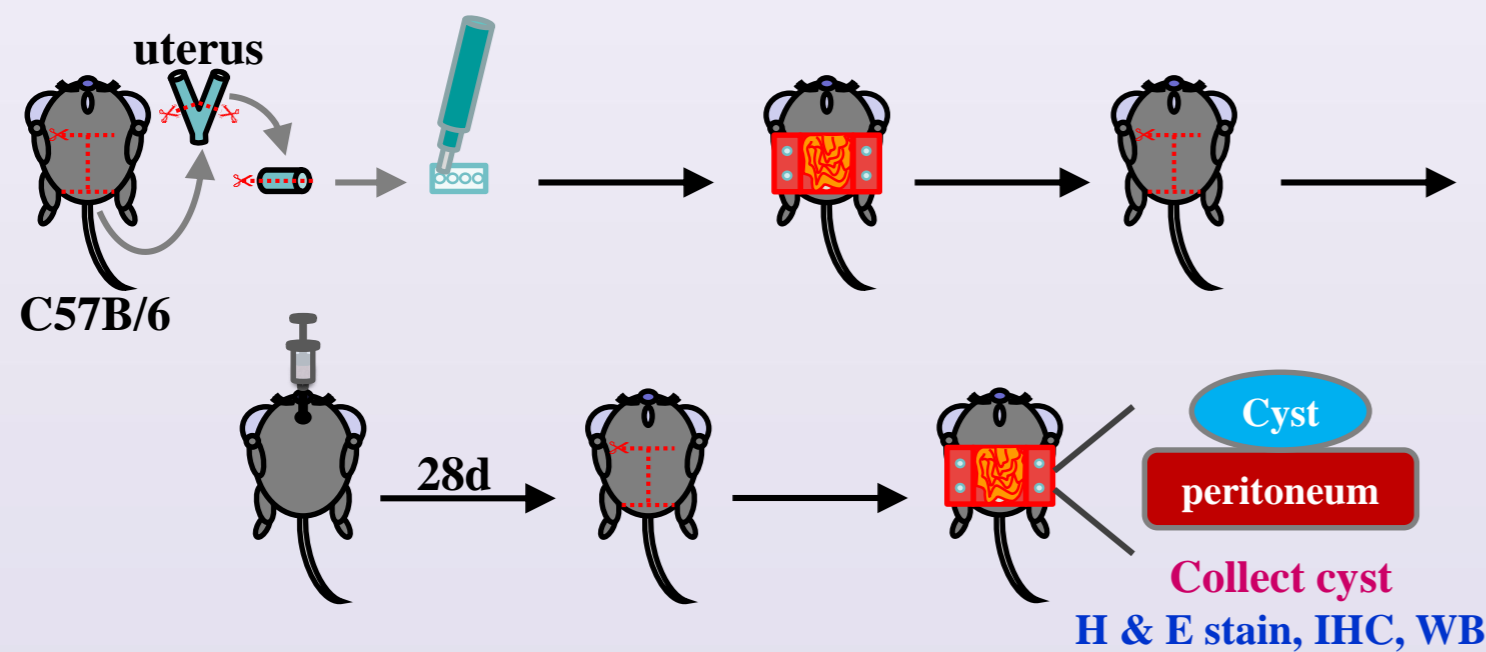
**Conclusions:** Consistent to the comprehension of TCM, the current study reveals Guizhi and Fuling are the most crucial components of GFW in treating endometriosis as they are the emperor drugs of this formula in TCM. Taoren is minister drug, while Shaoyao and Moutanpi are assistant drugs.

BACKGROUND

Endometriosis, a common painful gynecological disease, occurs in 10% of reproductive age women, 60% of women with pelvic pain, and 30% to 50% of infertile women.<sup>1</sup> Endometriotic patients usually suffer from dysmenorrhea, dyspareunia, infertility, chronic pelvic pain as well as symptoms associated with gastrointestinal and urinary tract involvement.<sup>2</sup> The etiology of endometriosis is commonly attributed to retrograde menstruation.<sup>3</sup> Since only a small percentage of women with retrograde menstruation develop endometriosis, additional modification of the immune system have been recently suggested to play a causative role in the pathogenesis of endometriosis.<sup>2,3</sup> Clinically, the aberrant cell-mediated and humoral immune responses were reported in both peripheral blood and peritoneal fluid of patients with endometriosis.<sup>4-6</sup> However, no impeccable theory can explain all cases of endometriosis. The current medical therapies, including non-steroidal anti-inflammatory drugs, hormone treatments, selective progesterone receptor modulators, selective estrogen receptor modulators, are common selections with obvious side effects and unpredictable outcomes in treating endometriosis.<sup>7-8</sup> Surgical interventions are usually used for patients complicated with endometrioma.

A novel treatment with different approach for endometriosis is required to improve the efficacy. Guizhi Fuling Wan (GFW) is a traditional Chinese herbal formula used to treat the disorder characterized by stagnation of qi and blood, such as endometriosis. In a Chinese herbal formula, each herb is designated as sovereign, minister, assistant or envoy drug which bears different importance in their therapeutic effects. For instance, Guizhi and Fuling are designated as sovereign drugs, while Taoren is a minister drug, while Shaoyao and Moutanpi are assistant drugs in GFW. In vivo, GFW was shown to induce apoptosis and inhibit proliferation in endometriotic tissue.<sup>9-11</sup> Consistent with other studies, our previous results also demonstrated the inhibition of endometriosis development by GFW.<sup>9-11</sup> Thus, this study aimed to demonstrate the relative importance of various herbs in GFW in treating endometriosis.

METHODS



- After sacrifice,
- ❖ The degree of pelvic adhesion and the size of endometriotic cysts were assessed.
  - ❖ The area of endometriotic cyst and the quantification of immunohistochemistry & Western blot (WB) were measured using NIH Image-J v1.53 DIA software.
  - ❖ The expression of ICAM-1 & Caspase-3 were demonstrated by IHC & WB.

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RESULTS

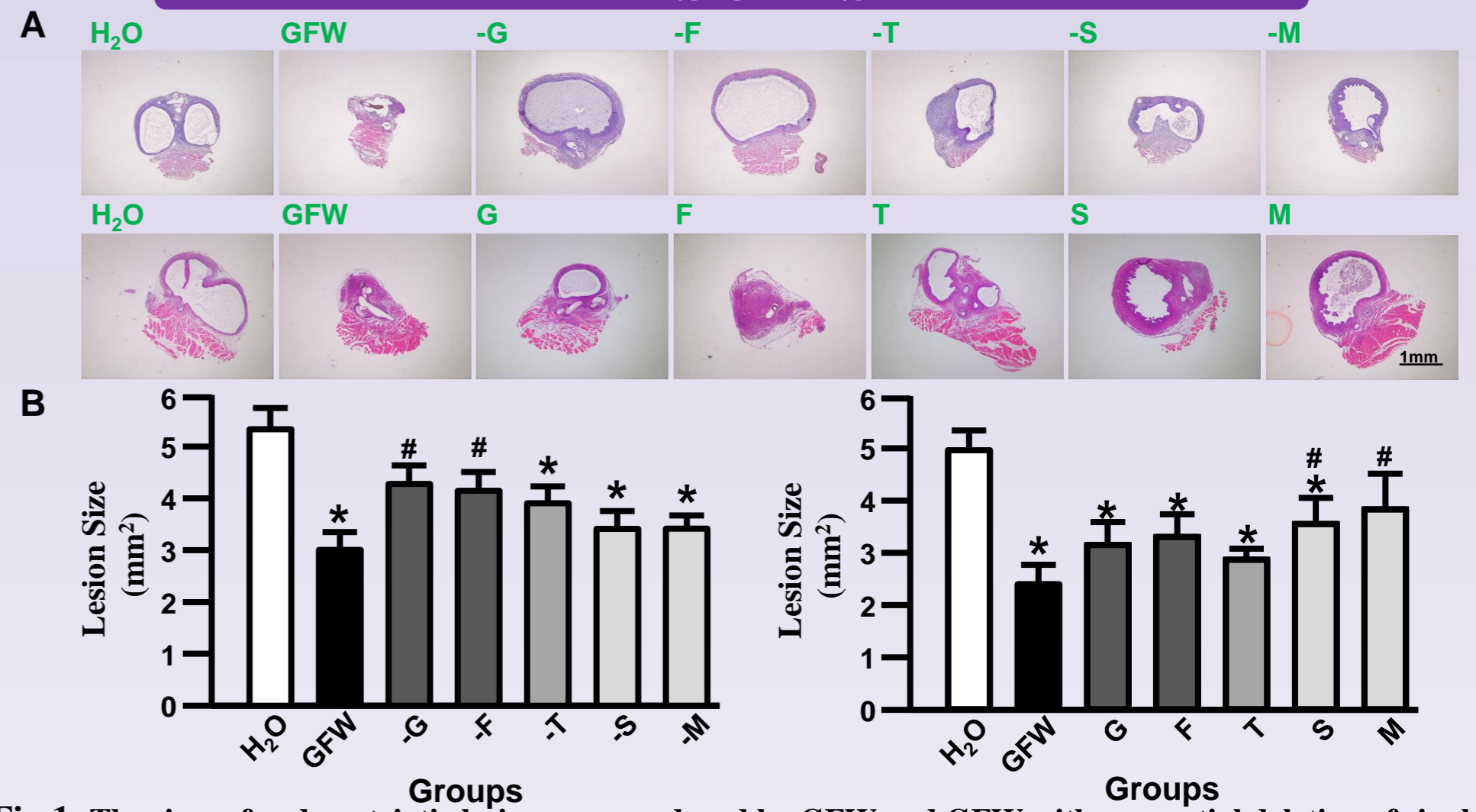


Fig 1. The sizes of endometriotic lesions were reduced by GFW and GFW with sequential deletion of single herb and signal herb for endometriosis. (A) Endometriotic lesions were collected and stained with eosin and hematoxylin. (B) The area of endometriotic cysts was measured by NIH ImageJ v1.53. The data were reported as mean ± SEM. n=5-9; \* comparison with H<sub>2</sub>O; # p < 0.05; # comparison with GFW; # p < 0.05. Scale bar: 1 mm.

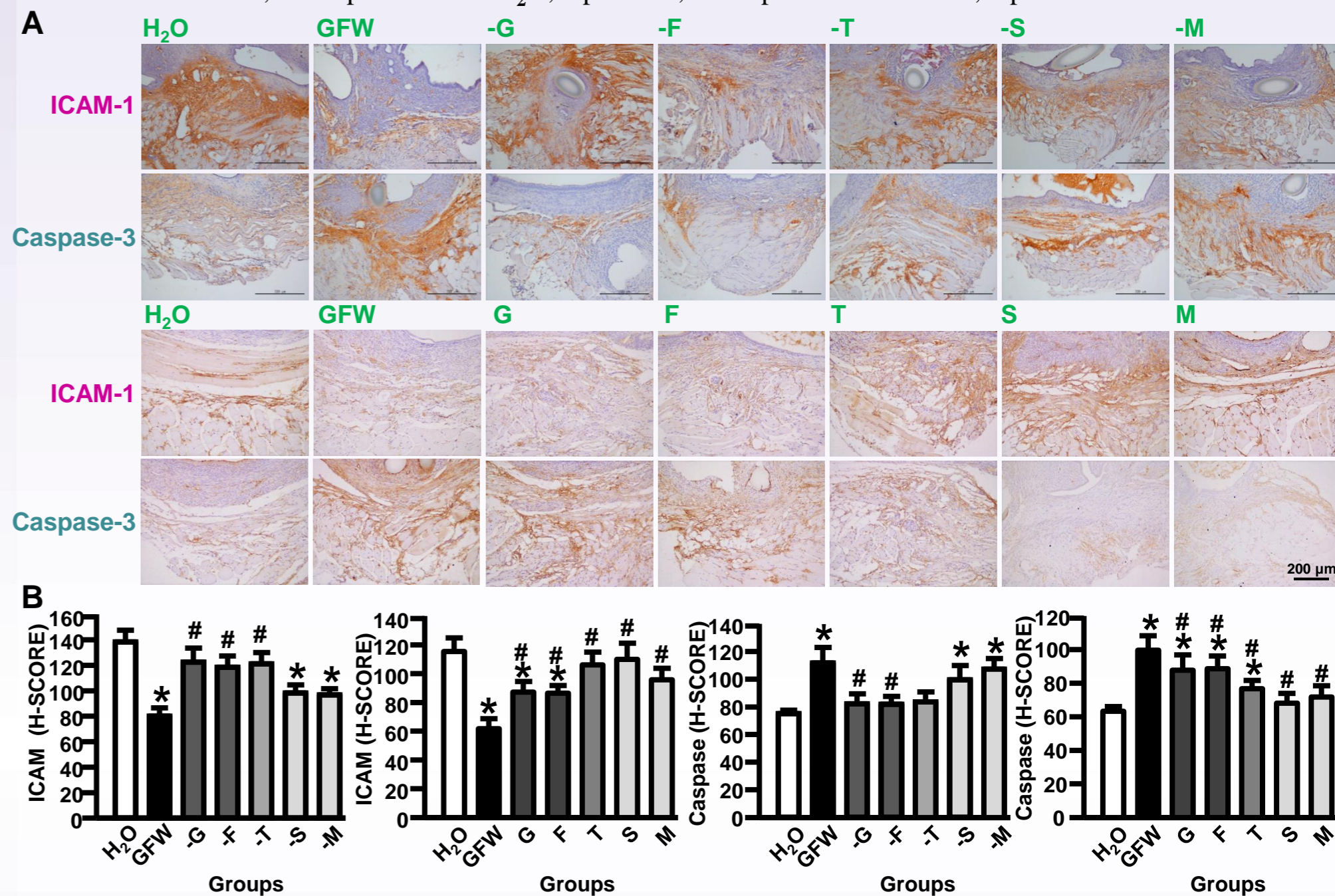


Fig 2. The IHC and protein expressions of ICAM-1 and Caspase-3 in endometriotic lesions were down-regulated by GFW and GFW with sequential deletion of single herb for endometriosis. (A) Endometriotic lesions were collected and stained with anti-mouse ICAM-1 and Caspase-3 primary antibody. The antigen-antibody complex was detected by DAB. (B) IHC showed ICAM-1 and Caspase-3 immunoreactivity was regulated by different treatments. The data were reported as mean ± SEM. N=5-9; \* comparison with H<sub>2</sub>O; # p < 0.05; # comparison with GFW; # p < 0.05. Scale bar: 200 µm.

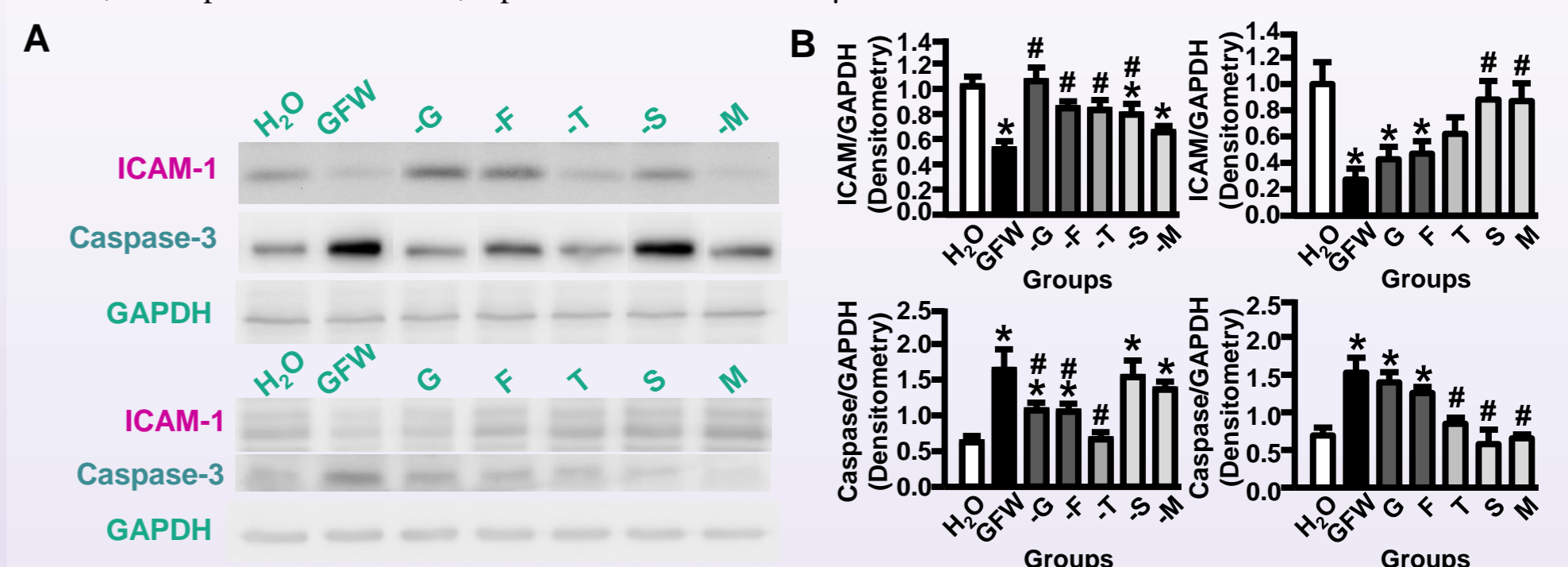


Fig 3. The protein expression of ICAM-1 & Caspase-3 in endometriotic lesions were regulated by GFW and GFW with sequential deletion of single herb for endometriosis. (A) Protein expressions were examined by Western blotting analysis. (B) Western blots were semi-quantitatively analyzed by densitometry of ICAM-1 and caspase-3. The data were reported as mean ± SEM. N=5-9; \* comparison with H<sub>2</sub>O; # p < 0.05; # comparison with GFW; # p < 0.05.

CONCLUSIONS

❖ This study verify the comprehension in traditional Chinese medicine which designates Guizhi and Fuling as sovereign drugs, Taoren as minister drug, and Shaoyao and Moutanpi as assistant drugs.

ACKNOWLEDGEMENTS

This study was supported by grants E-Da Hospital Research grants EDAH111057 (CCC), EDAH110012 (CYH), EDPJ110066 (SJH), EDAH110004 (SJH).

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