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In vivo Anti-Ulcerogenic Activity of *Equisetum telmateia* Ehrh. Extracts Used in Turkish Folk Medicine

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Abstract: The aerial part of *Equisetum telmateia* Ehrh. is used against various conditions including stomachache and peptic ulcer in Turkish folk medicine. In order to prove the claimed anti-ulcerogenic potential of the plant, aqueous and methanol extracts were prepared from aerial parts of *E. telmateia* and investigated for their anti-ulcerogenic effect on ethanol-induced gastric ulcer model in rats. Pharmacological experiments clearly demonstrated that the oral administration of both methanol and aqueous extracts of *Equisetum telmateia* Ehrh. plant showed significant stomach protection (77.9% and 100% ulcer inhibition, respectively) against the applied model of ulcerogenesis ($P < 0.001$). Since both extracts were found significantly active, 80% ethanol was preferred for the extraction of the plant material and this crude methanolic extract was further fractionated by successive solvent extractions with chloroform and H_2O saturated n-BuOH to obtain the chloroform and n-BuOH extracts as well as the remaining aqueous extract. The 80% ethanol extract and the solvent extracts were then submitted to pharmacological assay using the same in vivo experimental ulcer model. All the extracts statistically showed potent anti-ulcerogenic effect on this model ($P < 0.001$). However, the remaining aqueous extract was found prominent (98.2% ulcer inhibition). Consequently, folkloric utilization of *E. telmateia* on peptic ulcer has been confirmed in the present study by using ethanol-induced experimental ulcer model in rats.

Key Words: *Equisetum telmateia*, Equisetaceae, Horsetail, Anti-ulcer activity, Ethanol-induced ulcerogenesis, Peptic ulcer

Türkiye'de Halk İlacı Olarak Kullanılan *Equisetum telmateia* Ehrh. Bitkisinin Ekstrelerinin In vivo Antiülserojenik Aktivitesi

Özet: *Equisetum telmateia* bitkisinin topraküstü kısımları Türkiye'de halk arasında mide ağrısı ve peptik ülser dahil olmak üzere çeşitli hastalıkların tedavisinde halk ilacı olarak kullanılmaktadır. *E. telmateia*'nın iddia edilen antiülserojenik etkisini incelemek için toprak üstü kısımlarının sulu ve metanollü ekstreleri hazırlanmış ve sıçanlarda etanolle oluşturulan gastrik ülser modelinde incelenmiştir. Farmakolojik deneyler materyalin metanollü ve sulu ekstrelerinin her ikisinin de oral yolla uygulandığında bu ülser modeli üzerinde midede anlamlı bir koruma sağladığını (ülser önleme oranı sırasıyla %77,9 ve %100) göstermiştir ($P < 0,001$). Her iki ekstrenin de aktif olması nedeniyle, materyalin %80 etanol ile ekstre edilmesi tercih edilmiş; ardından %80 etanol ekstresi kloroform ve suyla doyurulmuş n-bütanol ile daha ileri bir ayırıma tabi tutularak, kloroform, n-bütanol ve kalan su ekstreleri elde edilmiştir. %80 etanol ekstresi ve sıvı-sıvı ekstraksiyonla elde edilen ekstreler aynı in vivo deneysel ülser modeline uygulanmıştır. Tüm ekstreler bu model üzerinde istatistiksel olarak kuvvetli antiülserojenik etki göstermiş ($P < 0.001$), ancak en belirgin etki kalan sulu ekstrede görülmüştür (ülser önleme oranı %98,2). Sonuç olarak bu çalışmayla *E. telmateia*'nın halk arasında peptik ülser üzerindeki kullanımı, sıçanlarda etanolle oluşturulan deneysel gastrik ülser modeli kullanılarak doğrulanmıştır.

Anahtar Sözcükler: *Equisetum telmateia*, Equisetaceae, Atkuyruğu, Antiülser aktivite, Etanolle oluşturulan ülser, Peptik ülser

Introduction

Traditional remedies are important sources for the discovery of new drug leads. A recent review reported that the anti-ulcerogenic potential of many plant remedies worldwide have been investigated experimentally so far and diverse molecules have been determined as the active ingredients (1). In Turkish folk medicine, several plant species have also been used to alleviate gastric complaints such as stomachache (2-7). A number of these plants have previously been evaluated experimentally for their anti-ulcerogenic potential (8-15).

The genus *Equisetum* (Equisetaceae) is diversely distributed in Anatolia and represented by 7 species in Turkish flora (16). All *Equisetum* species comprises an important biological resource in Turkish folk medicine against various complaints. Among these species *E. telmateia* has a prominent place in Turkish folk medicine. It has been used against diverse ailments in Anatolia as

expectorant and diuretic; to pass kidney stone or sand; against other urinary disorders, such as pyelonephritis, prostatic hypertrophy, or cystitis; for hemorrhoids, asthma; against stomachache or peptic ulcers; for eczema, acne, rheumatism; against pain in broken bones; to strengthen hair, skin, and nails, for infection in the mouth; for fungal and parasitic infections; and, against cardiac deficiency and arteriosclerosis (7,17-28).

In the ethnobotanical reports on Turkish folk medicine it has been reported that the aerial part of *E. telmateia* was used against stomachache and peptic ulcers by the local people in Pehlivanhoca village (Gönen-Balıkesir), Kızılcaköy village (Şile-İstanbul), and Trabzon (23,24,28). However, anti-ulcerogenic potential of *E. telmateia* has not been evaluated so far. In the present study, we aimed to study the anti-ulcerogenic activity of the extracts and fractions prepared from *E. telmateia* against an ethanol induced ulcerogenesis model in rats.

Materials and Methods

Plant Material

Aerial parts of *E. telmateia* Ehrh. was collected from the Uskumruköy, Kilyos-Istanbul (April 23, 2002) and the plant material was dried under shade. Identification of the voucher specimens was performed by Prof. Dr. Şinasi Yıldırımlı (Department of Botany, Faculty of Science, Hacettepe University). Herbarium specimens (GUE-2609) are stored in the Herbarium of Gazi University (Ankara, Turkey).

Extraction and Fractionation

Extraction of the aerial parts of E. telmateia with methanol: 10 g of dried aerial parts of *E. telmateia* was extracted with MeOH (2 × 100 ml) at room temperature for overnight. The filtered and combined extract was concentrated to dryness under reduced pressure to give 705 mg of MeOH extract (7.05% yield).

Extraction of the aerial parts of E. telmateia with water: 150 ml of boiling H_2O was added on 10 g of dried aerial parts of *E. telmateia* and kept stirred on a magnetic stirrer adjusted to 80 °C for 20 min and then lyophilized to give 1584 mg of aqueous extract (15.84% yield).

Extraction of aerial part of E. telmateia with 80% ethanol: Dried aerial parts of the plant (770 g) were extracted with 80% EtOH (4 × 6 l) in a percolator at room temperature for 1 week. The filtered and combined extract was concentrated to dryness under reduced pressure to give 99.52 g of 80% EtOH extract (12.92% yield).

Fractionation of the 80% ethanol extract through solvent extraction: An aliquot of the 80% ethanol extract (89.52 g) was diluted in 300 ml distilled water and extracted with $CHCl_3$ (10 × 0.25 l), and H_2O-saturated *n*-BuOH (15 × 0.25 l) successively, in a separatory funnel. Each extract and the remaining aqueous part were then evaporated to dryness under reduced pressure to give $CHCl_3$ extract (19.61 g, 21.09% yield), *n*-BuOH extract (16.78 g, 18.74% yield), and remaining aqueous extract (34.02 g, 38.00% yield).

Pharmacological Experiments

Animals: Spraque-Dawley rats of either sex (120-220 g) were purchased from the Animal Breeding Laboratories of Gülhane Military Academy of Medicine (Ankara, Turkey). The animals were left for at least 7 days for acclimatization to animal room conditions (24 °C) before experiments, maintained on a standard pellet diet, and supplied water ad libitum. The food was withdrawn 24 h before the experiment, but the animals were allowed free access to water. To avoid coprophagy, the rats were fasted in wire-bottomed cages. Six rats were used for each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals as well as the rules of the Gazi University Animal Ethic Committee.

Preparation of test samples for bioassay: Test samples were given orally to the animals in a 7 ml/kg body weight dosage after suspending in 0.5% Tween 80 in distilled water. Control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with administration of appropriate volumes of the dosing vehicle. A cytoprotective drug, misoprostol (400 mg/kg, per os), was used as a reference compound.

Effects on ethanol-induced ulcerogenesis: The method described by Robert et al. was employed with modifications (29). The test sample was administered orally 30 min before the oral application of ethanol 96% (1 ml) to a group of 6 rats. Sixty min later, the animals were sacrificed with an over-dose of diethyl ether. The abdomen of each rat was dissected and the stomach was slightly taken out. Then the esophagus was tied as a knot nearest the cardia by a surgical suture. From the duodenum side, 10 ml of 10% formalin solution was injected into the stomach. The distended stomach was

immediately tied on the pyloric sphincter using another surgical suture to avoid leakage of the formalin solution. Finally the distended stomach was removed from the abdomen, inflated with 10 ml of 10% formalin solution and immersed in the same solution to fix the outer layer of the stomach. Each stomach was then dissected along the greater curvature, rinsed with tap water to remove gastric contents and blood clots, and examined under a dissecting microscope (20 × 6.3) to assess the formation of ulcers. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the inhibition percentage was calculated by the following formula:

Inhibition % = [(UI control- UI treated) / UI control] × 100.

Statistical analysis of data: Results were expressed as mean ± S.E.M. The statistical difference between the mean ulcer index of the treated group and that of the control was calculated by ANOVA and Student-Neuman-Keuls multiple comparisons test.

Results and Discussion

According to general experimental procedures, aqueous and methanol extracts of the aerial parts were prepared initially for the preliminary activity assessment and both extracts were found to be effective against the ethanol-induced ulcer model in rats. Since the administration dose of the plant material as a remedy was not described in folkloric notes, we estimated the doses of the extracts as per kg of body weight depending on the yields obtained from 10 g of dried ground plant material. Thus, aqueous and MeOH extracts of the plant material were administered to rats in 705 and 1584 mg/kg doses, respectively. As shown in Table 1, both extracts demonstrated potent gastroprotective effects ($P < 0.001$) against ethanol-induced experimental gastric ulcer model; however, the potency of the aqueous extract was found to be higher and this extract provided a total protection from gastric lesions (Figure 1). Although the gastroprotective effect of MeOH extract was found to be less potent (77.9% ulcer inhibition), the stomachs of 2 out of six rats were completely protected from any visible damage.

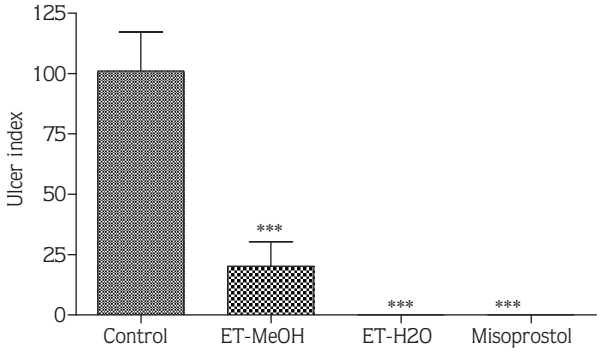
Moreover, despite the higher activity of aqueous extract comparing to that of the MeOH extract, both extracts were significantly ($P < 0.001$) active. Accordingly, both polar and less polar components of the aerial parts are supposed to be effective against ethanol-induced ulcerogenesis. In order to extract both groups of components in the plant material, we employed an 80% ethanol extraction process. As shown in Table 1, the 80% ethanol extract of *E. telmateia* also provided a potent inhibition (96.4% inhibition), 4 stomachs out of 6 examined were completely protected from any visible

Table 1. Gastroprotective effects of extracts and fractions of *E. telmateia* on EtOH-induced ulcerogenesis in rats.

Material	Dose (mg/kg)	Ulcer Index (Mean ± S.E.M.)	Prevention from ulcer[a]	Ulcer inhibition (%)
Control	-	101.1 ± 16.1	-	-
MeOH extract	705	20.2 ± 10.2 ***	2/6	77.9
Aqueous extract	1584	0.0 ± 0.0 ***	6/6	100.0
Misoprostol	0.4	0.0 ± 0.0 ***	6/6	100.0
Control	-	182.8 ± 19.2	-	-
80% EtOH extract	1292	6.8 ± 5.5 ***	4/6	96.3
$CHCl_3$ extract	719	55.2 ± 17.4 ***	1/6	69.8
n-BuOH extract	615	43.8 ± 18.8 ***	1/6	76.0
Remaining H_2O extract	1248	3.2 ± 3.2 ***	5/6	98.2

*** : $P < 0.001$

[a]: Number of rats in which the bleeding of the stomach was prevented.

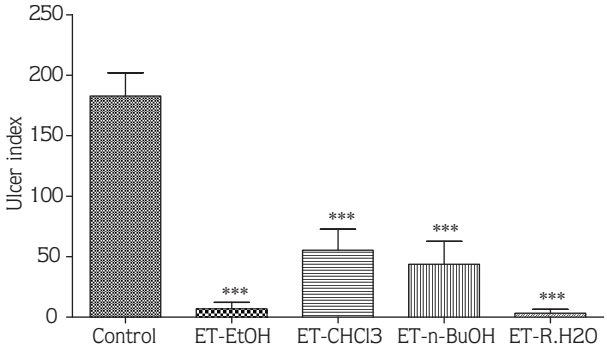


*** : P < 0.001

ET-MeOH:E. telmateia methanol extract

ET-H_2O: E. telmateia aqueous extract

Figure 1. Gastroprotective effect of aqueous and methanol extracts of *Equisetum telmateia*.



*** : P < 0.001

ET-EtOH: E. telmateia 80% ethanol extract

ET-$CHCl_3$: E. telmateia chloroform extract

ET- n-BuOH: E. telmateia n-BuOH extract

ET-R.H_2 O: E. telmateia remaining aqueous extract

Figure 2. Gastroprotective effect of 80% ethanol, chloroform, n-BuOH, and remaining water extracts of *Equisetum telmateia.*

damage. This extract was then subjected to successive solvent extractions according to the general bioassay-guided fractionation protocol employed in our previous studies (30) to give $CHCl_3$ extract, *n*-BuOH extract, and the remaining H_2O extract. These subextracts were then orally administered to rats in doses estimated from their yields and their effects were tested using the same gastric ulcer model. All subextracts showed significant ($P < 0.001$) anti-ulcerogenic activities against EtOH-induced ulcer model on rats (Figure 2); however, the effect of the remaining H_2O extract was the highest (98.2% ulcer inhibition) one, and 5 out of 6 stomachs were totally protected from any visible damage (Table 1).

In a reference survey, only few records have been found on the biological effects and chemical composition of *E. telmateia*. Geiger et al. (31) isolated some flavonoids from the plant and determined their structures as kaempferol 3- -D-(6-O-acetylglucoside)-7- -D-glucoside and kaempferol 3- -D-(6-O-acetylglucoside)-7- -L-rhamnoside, while Baytop and Gürkan (32) reported the existence of alkaloids with a qualitative test from *E. telmateia*. The antimicrobial and potent antioxidant activity of the polar extracts of *E. telmateia* was reported by several groups (33-35), while Milavanovic et al. (34) also reported its genotoxicity. Szelenyi and Brune (36) suggested that antioxidant compounds may have a preventing role against the damage in gastric mucosa and Oka et al. (37) demonstrated the protective effect of some radical scavengers against mucosal injuries. Consequently, the results suggested that the anti-ulcerogenic activity of *E. telmateia* extracts and subextracts could be due, at least in part, to the presence of compounds with antioxidant activity.

In conclusion, the present study has clearly demonstrated that *E. telmateia* possesses a potent anti-ulcerogenic activity as suggested in Turkish folk medicine. This is the first report on the anti-ulcerogenic activity of *E. telmateia*; however, further studies might be necessary on the isolation and mechanism of the active component(s).

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References

1. Yeşilada E, Gürbüz I. A compilation of the studies on the anti-ulcerogenic effects of medicinal plants. In: Singh S, Singh VK, and Govil JN. ed. Recent Progress in Medicinal Plants. Vol.II. Phytochemistry and Pharmacology. SCI Tech Publishing LLC; 2003: pp. 111-174.
2. Baytop T. Therapy with medicinal plants in Turkey, past and present. Nobel Tıp Kitapevi. Istanbul; 1999.
3. Honda G, Yeşilada E, Tabata M et al. Traditional medicine in Turkey VI. Folk medicine in West Anatolia: Afyon, Kütahya, Denizli, Muğla, Aydın provinces. J Ethnopharmacol 53: 75-87, 1996.
4. Sezik E, Zor M, Yeşilada E. Traditional medicine in Turkey II. Folk medicine in Kastamonu. Int J Pharmacog 30: 233-239, 1999.
5. Sezik E, Yeşilada E, Honda G et al. Traditional medicine in Turkey X. Folk medicine in Central Anatolia. J Ethnopharmacol 75: 95-115, 2001.
6. Tabata M, Honda G, Sezik E. A report on Traditional Medicine and Medicinal Plants in Turkey (1986). Faculty of Pharmaceutical Sciences, Kyoto University. Japan; 1988.
7. Yeşilada E, Honda G, Sezik E et al. Traditional medicine in Turkey V. Folk medicine in inner Taurus Mountains. J Ethnopharmacol 46: 133-152, 1995.
8. Gürbüz İ, Akyüz Ç, Yeşilada E et al. Anti-ulcerogenic effect of *Momordica charantia* L. fruits on various ulcer models in rats. J Ethnopharmacol 71: 77-82, 2000.
9. Gürbüz İ, Üstün O, Yeşilada E et al. In vivo gastroprotective effects of five Turkish folk remedies against ethanol-induced lesions. J Ethnopharmacol 83: 241-244, 2002.
10. Gürbüz İ, Üstün O, Yeşilada E et al. Anti-Ulcerogenic activity of some plants used as folk remedy in Turkey. J Ethnopharmacol 88: 93-97, 2003.
11. Gürbüz İ, Özkan AM, Yeşilada E et al. Anti-Ulcerogenic activity of some plants used in folk medicine of Pınarbaşı (Kayseri-Turkey). J Ethnopharmacol 101: 313-318, 2005.
12. Gürbüz İ, Yeşilada E. Evaluation of the anti-ulcerogenic effect of sesquiterpene lactones from *Centaurea solstitialis* L. ssp. *solstitialis* by using various in vivo and biochemical techniques. J Ethnopharmacol 112: 284-291, 2007.
13. Yeşilada E, Gürbüz İ, Shibata H. Screening of Turkish anti-ulcerogenic folk remedies for anti-*Helicobacter pylori* activity. J Ethnopharmacol 66: 289-93, 1999.
14. Yeşilada E, Gürbüz İ, Bedir E et al. Isolation of anti-ulcerogenic sesquiterpene lactones from *Centaurea solstitialis* L. ssp. *solstitialis* through bioassay-guided fractionation procedures in rats. J Ethnopharmacol 95: 213-219, 2004.
15. Yeşilada E, Gürbüz İ. Evaluation of the anti-ulcerogenic effect of the flowering herbs of *Hypericum perforatum*. J Fac Pharm Gazi 15: 77-83, 1998.
16. Cullen J. Pteridophyta. In: Davis PH. ed. Flora of Turkey and The East Aegean Islands. Vol 1. Edinburg University Press; 1997: pp. 31-34.
17. Akalın E, Alpınar K. An investigation on medicinal and edible wild plants of Tekirdağ. Ege Üniv Ecz Fak Der 2: 1-11, 1994.
18. Ertuğ F. Bodrum yöresinde halk tıbbında yararlanılan bitkiler. In: Başer KHC and Kırımer N. ed. 14. Bitkisel İlaç Hammaddeleri Toplantısı; 2004.
19. Ezer N, Arısan ÖM. Folk medicines in Merzifon (Amasya, Turkey). Turk J Bot 30: 223-230, 2006.
20. Gülay EG, Özhatay N. An ethnobotanical study in Çatalca (European part of Istanbul) II. Turkish J Pharm Sci 3: 73-89, 2006.
21. Koçyiğit M, Özhatay N. Wild plants used as medicinal purpose in Yalova (Northwest Turkey). Turkish J Pharm Sci 3: 91-103, 2006.
22. Kültür Ş. Medicinal plants used in Kırklareli Province (Turkey). J Ethnopharmacol 111: 341-364, 2007.
23. Tuzlacı E, Aymaz PE. Turkish folk medicinal plants, Part IV: Gönen (Balıkesir). Fitoterapia 72: 323-343, 2001.
24. Tuzlacı E, Tolon E. Turkish folk medicinal plants, Part III: Şile (İstanbul). Fitoterapia 71: 673-685, 2000.
25. Uğurlu E, Seçmen Ö. Medicinal plants popularly used in the villages of Yunt Mountain (Manisa-Turkey). Fitoterapia 79: 126-131, 2007.
26. Uzun E, Sarıyar G, Adsersen et al. Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species. J Ethnopharmacol 95: 287-296, 2004.
27. Yeşilada E, Sezik E, Honda G et al. Traditional medicine in Turkey IX. Folk medicine in north-west Anatolia. J Ethnopharmacol 64: 195-210, 1999.
28. Yazıcıoğlu A, Tuzlacı E. Folk medicinal plants of Trabzon (Turkey). J Pharm Univ Mar 11: 333-342, 1995.
29. Robert A, Nezamis JE, Lancester C et al. Cytoprotection by prostaglandins in rats. Gastroenterology 77: 433-43, 1979.
30. Yeşilada E, Gürbüz İ, Ergun E. Effects of *Cistus laurifolius* L. flowers on gastric and duodenal lesions. J Ethnopharmacol 55: 201-211, 1997.
31. Geiger H, Lang U, Britsch E et al. Flavonol glycosides from *Equisetum telmateia*. Phytochemistry 17: 336-337, 1978.
32. Baytop T, Gürkan E. Pharmacognostical investigations on some of the *Equisetum* species (*E. palustre, E. ramosissimum, E. telmateia*) of Turkey. J Fac Pharm Istanbul 8: 63-74, 1972.
33. Correia H, Gonzalez-Paramas A, Teresa AM et al. Characterization of polyphenols by HPLC-PAD-ESI/MS and antioxidant activity in *Equisetum telmateia*. Phytochem Anal 16: 380-387, 2005.
34. Milovanovic V, Radulovic N, Todorocic Z et al. Antioxidant, antimicrobial and genotoxicity screening of hydro-alcoholic extract of five Serbian *Equisetum* species. Plant Food Hum Nut 62: 113-119, 2007.
35. Stajner D, Popovic BM, Canadanovic-Brunet J et al. Free radical scavenging activity of three *Equisetum* species from Fruska gora mountain. Fitoterapia 77: 601-604, 2006.
36. Szelenyi I, Brune K. Possible role of oxygen free radicals in ethanol-induced gastric mucosal damage in rats. Dig Dis Sci 33: 865-871, 1988.
37. Oka S, Ogino K, Hobara T et al. Effects of various mucosal protective drugs on diethyldithiocarbamate-induced antral ulcer in rats. Eur J Pharmacol 197: 99-102, 1991.