Hypericum perforatum extracts healed gastric lesions induced by hypothermic restraint stress in Wistar rats

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ABSTRACT

الأهداف: لمعرفة التأثيرات والنتائج لـ-*Hypericum perforatum* HP في على علاج تقرحات غشاء المعدة الناتج عن توتر الانخفاض الحراري (HRS).

الطريقة: أُجريت دراسة تم فيها استخدام 60 فأر غربياً تتراوح أوزانها مابين 200–250gm. قسمت الفئران إلى ستة مجموعات، عدا اثنين أُبقيت كمجموعة تحكم. بعد حدوث توتر الانخفاض الحراري (HRS) تم تثبيت الفئران على لوح خشبي وإبقائهم لمدة 3 ساعات تحت درجة حرارة 4°C بعد إبقائهم بلا طعام لمدة 36 ساعة. بعد حدوث توتر الانخفاض الحراري تم إعطاء جرعات من (HPEs) تراوح مابين (25–50–100mg/kg) في اليوم ولمدة 3 أيام عن طريق الفم لثلاثة مجموعات. كما تم إعطاء حقن رانيتدين (Ranitidine) تحت الجلد للمجموعة أخرى كمجموعة تحكم ايجابي (Positive Control). تم تقييم التلف في أغشية المعدة عن طريق التحليل المجهري (Microscopic) والنظري (Macroscopic) بعد نهاية فترة العلاج. أُجريت هذه الدراسة في قسم علم حياء – جامعة دوملوبنار – كوتهيا – تركيا، خلال الفترة مابين مارس2006م وحتى يوليو لعام 2006م.

النتائج: أثبت التحليل النظري أن العلاج بـ(25–50–100mg/kg) في اليوم بعقار (HPEs) يؤدي إلى التئام ملحوظ لقرحة جدار المعدة بالمقارنة مع مجموعات التحكم (Control) بنسب تتراوح بين (75%، 95%، 65%) على التوالي (p=0.001)، أيضاً: العلاج بالرانيتدين (Ranitidine) حقق التئام ملحوظ بالمقارنة مع مجموعة التحكم (Control). كما أثبت التحليل المعملي (Histopathology) إن العلاج بجرعة 50mg/kg HP من يقلل من تقرحا جدار المعدة بطريقة ملحوظة بالاعتماد على الجرعة المذكورة. من المقترح أن يلعب الـ(HPEs) دوراً هاماً في علاج تقرحات المعدة.

Objectives: To investigate the healing effects of *Hypericum perforatum* (HP) on gastric mucosal damage induced by hypothermic restraint stress (HRS).

Methods: Sixty Wistar breed rats of 200-250 gm were used in this study carried out at the Biology Department of Dumlupinar University, Kutahya, Turkey in 2006. The animals were divided into 6 groups, 2 of which were controls. The HRS were induced by strapping the rats on a wooden plank and keeping them for 3 hours at 4°C after a starvation period of 36 hours. After HRS, 25, 50, and 100 mg/kg/day *Hypericum perforatum* extracts (HPEs) were orally administrated to the 3 groups during the 3-day treatment. Fifty mg/kg ranitidine was administered everyday as subcutaneous injection to a group selected as a positive control. At the end of treatment, lesions in the stomach were evaluated macroscopically and microscopically.

Results: Macroscopic analyses showed that treatment with HPEs 25, 50, and 100 mg/kg/day significantly healed lesions compared to control groups by 65, 95, and 75% (p=0.001). Treatment with ranitidine also healed ulcers significantly compared with the control groups. Histopathologic analyses indicated that 50 mg/kg/day HP produced the most significant effect.

Conclusion: Moderate doses of HP produced significant healing of HRS induced gastric ulcer in rats. The present study indicated that HPEs have therapeutic potential for the control of ulcers.

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Hypericum perforatum L. (Hypericaceae Syn. Guttiferae) extracts have been widely used in traditional medicine for centuries for the treatment of trauma, burns, rheumatism, neuralgia, gastroenteritis, peptic ulcers, hysteria, bed-wetting, depression, bruises, swelling, inflammation, anxiety, bacterial, and viral infections.[1,2] Its wound healing, antiseptic, antioxidant,

hepatoprotective, antispasmodic, antibiotic, and antidepressant properties have been demonstrated.[3,4] In recent years, leaves and flowers of *Hypericum perforatum* (HP) became popular because of their antidepressant effects.[5,6] Hypericum contains at least 10 classes of biologically active compounds; these are, naphthodianthrones, phloroglucinols, flavonoids, procyanidins, tannins, essential oil, amino acids, phenylpropanes, xanthones, and other water-soluble components (organic acids, peptides, polysaccharides, and so forth).[6] Several plants containing alkaloids, flavonoids, and tannins have been shown to possess several biological properties such as antioxidant effects.[7] *Hypericum perforatum* is reported to contain high amounts of these compounds, especially flavonoids.[6,8] Flavonoids are known as a natural compounds having antiulcerogenic effects.[9-11] El-Sherbiny et al[12] reported that low doses of *Hypericum perforatum* extract (HPE) demonstrated antioxidant activity. Abdel-Salam[13] reported that administration of HP inhibits gastric acid secretion in pyloric-ligated rats, and exacerbated gastric lesions caused by indomethacin. It was reported that using HP as an antidepressant might cause gastrointestinal irritations.[8] Despite some reports on gastric adverse effects of HPEs, they have been commonly used in the treatment of gastric disorders in Turkey.[14-16] Our aim was thus, to determine the effects of various dosages of HPE on gastric ulcers induced by hypothermic restraint stress (HRS) in rats.

Methods. This study was carried out between March 2006 and July 2006 at the Physiology Laboratory of the Biology Department of the Science and Arts Faculty, and the Pathology Laboratory of the Faculty of Medicine, Dumlupinar University, Kutahya, Turkey.

Collection of the herbs. Samples of *Hypericum perforatum L.* were collected during the flowering period from the steppe fields of Mandal Hill in the Tasoluk Region, Afyonkarahisar, Turkey. A voucher specimen (Akcicek 1787) was deposited in the herbarium of Necatibey Education Faculty, Balikesir University, Balikesir, Turkey.

Preparation of the extract. Air-dried aerial parts of the HP were powdered and extracted with 80% methanol by stirring at room temperature for 24 hours. The extract was filtered, concentrated, and evaporated to dryness (yield 27.4%). The dried extract was suspended in distilled water and used for further studies. The phytochemical screening of the extract with liquid chromatography-mass spectrometry techniques revealed the presence of hypericin, hyperforin, flavonoids, and tannins.

Animals. Albino Wistar rats weighing 200-250 gm of either gender, obtained from the central animal house of Dumlupinar University were used for the study. Rats were housed individually in cages, maintained under standard conditions (12 hours light:12 hours dark cycle; 25±3ºC) and were fed with standard pellet and water ad libitum. Animal studies were performed after approval from the Animal Care and Ethics Committee of Dumlupinar University.

Hypothermic-restraint stress (HRS) induced gastric ulcers. The animals were randomly divided into 6 groups consisting of 10 rats each. Groups 1 and 2 represent controls, which received only HRS, groups 3-5 received HPE orally at the doses of 25, 50, and 100 mg/kg body weight. Group 6 received ranitidine subcutaneously at a dose of 50 mg/kg body weight for positive control.

Gastric lesions were produced according to the method modified slightly from Demirbilek et al.[17] The rats were deprived of food for 36 hours with free access to water and kept in raised mesh-bottom cages to prevent coprophagy prior to the experiment. The HRS stress was given by strapping the rats on a wooden plank and keeping them at 4ºC for 3 hours. After HRS administration, the rats in group one were sacrificed by cervical dislocation to demonstrate gastric lesions. Group 2 received nothing during the experiment, and was used as a control of healing to demonstrate ulcers that did not improve spontaneously during the treatment period. One day after HRS, groups 3-5 received 25, 50, and 100 mg/kg/day HPE orally. The rats in group 6 received 50 mg/kg ranitidine subcutaneously everyday. After 3 days of treatment all animals were sacrificed by cervical dislocation. The stomach was taken out, and cut open along the greater curvature and the grade of lesions was scored by a person unaware of the experimental protocol in the glandular part of the stomach with the help of a magnifying glass and a millimeter scale as described by Das and Banerjee[18] according to the following criteria: 0 = no pathology; 1 = a small 1-2 mm ulcer; 2 = medium 3-4 mm ulcer; 4 = a large 5-6 mm ulcer; 8 = a larger >6 mm ulcer. The sum of the total severity scores in each group of rats divided by the number of animals was expressed as the mean ulcer index.[18]

Histopathological studies. After macroscopic evaluation, stomachs were preserved in 10% formaldehyde solution for histopathological studies. In each specimen, paraffin sections of 4 μm in thickness were obtained, stained with hematoxylin and eosin and then assessed by the same person who was unaware of the experimental procedure under light microscopy for severity of histopathological changes such as congestion, edema, hemorrhage, and necrosis, expressed on an arbitrary scale[19] as per the following criteria: - : normal; + : little effect; ++ : appreciable effect; +++ : severe effect; ++++ : very severe effect. Ulcer injury was scored by the same person as described by Takeuchi et al[20] according

to the following criteria: 1 = no damage; 2 = shallow damage not exceeding 25% of the mucosal depth; 3 = moderate damage reaching beyond 25% of the mucosal depth, but not exceeding 75%; 4 = deep damage reaching 75% of the mucosal depth. The ulcer depth index was calculated by dividing the mean mucosal thickness by mean ulcer depth. The ulcerated portion was measured as percentage size of the ulcer in 3 mm of gastric mucosa.[21]

Statistical analysis. The results were expressed as mean ± S.E.M. and statistical differences between several treatments, and their respective control was determined by one-way analysis of variance (ANOVA), followed by Fisher's least significant difference tests using the Statistical Package for Social Sciences version 14.0 software (SPSS Inc., Chicago, IL., USA). The level of significance was set at $p<0.05$.

Results. ***Macroscopic studies.*** The macroscopic observation showed that HRS induced multiple gastric mucosal lesions, mostly 3-4 mm in size with bleeding during the observation in control groups. The mean ulcer indices of control I was 23 ± 2.90, and of control II was 20 ± 2.47. There was no significant difference between the control groups. Treatment with HPE 25, 50, and 100 mg/kg/day significantly healed lesions compared to control II group by 65, 95, and 75% (**Figure 1**) (p=0.001). Treatment with ranitidine 50 mg/kg/day subcutaneously also healed ulcers significantly by 85% as compared with control II (p=0.001). Compared to 25 mg/kg/day HPE, 50 mg/kg/day HPE significantly healed lesions (p=0.05). There was no significant difference in ulcer indices between other treatment groups (**Figure 1**).

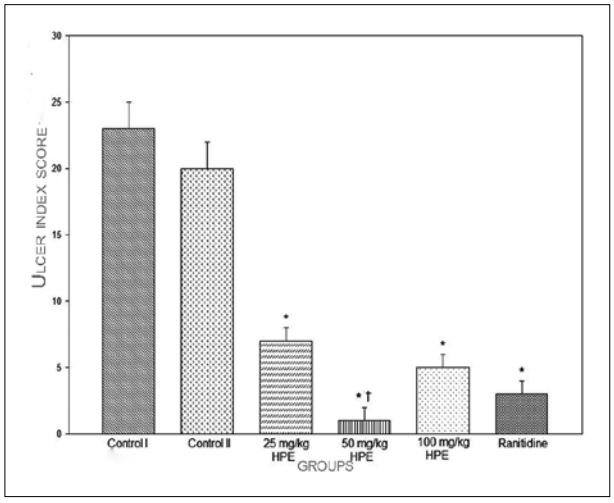

Figure 1 - Effects of *Hypericum perforatum* extract (HPE) treatment on gastric lesions induced by hypothermic restraint stress (ulcer index). Statistical significance: *p=0.001 versus controls, †p=0.05 versus 25 mg/kg HPE-group.

Histopathological studies. Microscopical data were consistent with macroscopic observation. The histomorphological studies showed that the lesions induced by HRS were revealed by acute, moderate, or deep erosive defects, which appeared with hemorrhage, congestion, edema, necrosis, and a small amount of cell infiltrations in the control groups (**Figure 2**). For the animal groups subjected to HPEs or ranitidine, regenerative improvements were observed in the mucosal lesions. However, some localized edema and congestion and, sometimes, superficial defects were rarely seen. In some cases, epithelial loss was also determined (**Figure 3**). In the ulcer depth index, there was no significant difference between control groups. Treatment with 25, 50, 100 mg/kg/day HPE and 50 mg/kg/day subcutaneous ranitidine significantly reduced ulcer depth compared to control II (p=0.001).

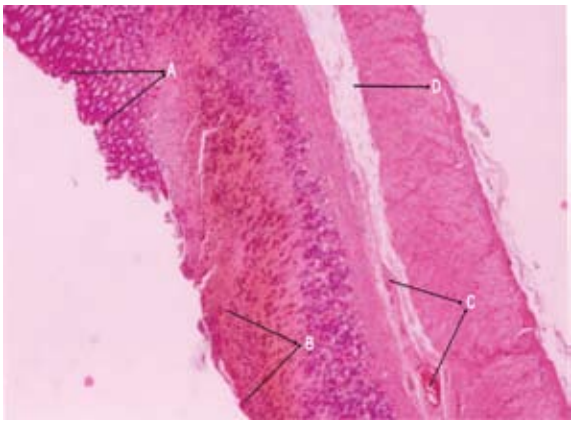

Figure 2 - The stomach wall of a control rat after treatment with hypothermic restraint stress. **a**) Hemorrhage, **b**) ulcer, **c**) congestion, and **d**) edema can be seen. (Hematoxylin & Eosin, x 40)

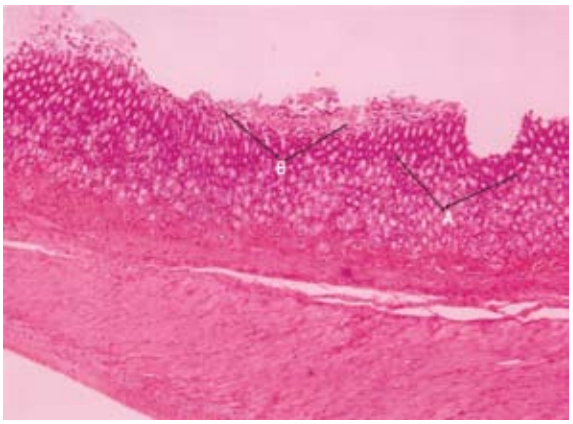

Figure 3 - The stomach wall of a rat after treatment of 50 mg/kg/day *Hypericum perforatum* extract. **a**) Congestion, and **b**) minimal erosion can be seen. (Hematoxylin & Eosin, x 40).

Table 1 - Histopathological examination of gastric lesion induced by hypothermic restraint stress.

Groups	Size of the ulcer in 3 mm of gastric mucosa (%)	Ulcer depth index	Congestion	Edema	Hemorrhage	Necrosis
Control I	80.3	1.24	++	+++	+++	+++
Control II	73.0	1.37	++	++	++	++
Ranitidine	32.9	3.04*†	+	+	-	-
HP (25 mg/kg)	42.9	2.33*	+	+	-	-
HP (50 mg/kg)	27.8	3.60*†	+	+	-	-
HP (100 mg/kg)	37.9	2.64*	+	+	-	-

HP - *Hypericum perforatum,* Statistical significance: *p<0.001 versus controls, †p=0.05 versus 25 mg/kg, and 100 mg/kg HP extract group. - : normal, +: little effect, ++: appreciable effect, +++: severe effect

The 50 mg/kg/day HPE, and ranitidine significantly decreased ulcer depth versus other HPE treated groups (p=0.05). There was no significant difference between 50 mg/kg/day HPE and the ranitidine group (**Table 1**).

Discussion. In the present study, gastric ulcer was induced by HRS in rats, and treated with different doses of HPEs; after the treatment period stomach tissue samples were evaluated macroscopically and microscopically. The methanol extract of HP at doses 25, 50, and 100 mg/kg/day produced significant healing in an HRS induced gastric ulcer model. From the above results, it was concluded that the present study seems to support the claims by traditional medicine practitioners on the usefulness of the aerial parts of HP for the treatment of ulcers. Various physical and psychological stresses cause gastric ulceration in human and experimental animals.[22] In rats, HRS causes gastric ulceration not only by increased acid secretion, disruption of mucosal barrier, reduction of gastric mucosal blood flow or inhibition of gastric mucus and bicarbonate secretion, but also by free radical formation.[17,23,24] Quercetin and rutin are the most important flavonoids of this herb. It has been reported that quercetin and rutin have antiulcerogenic effects through mechanisms such as free radical scavenging, decreasing of histamine secretion from mast cells, increasing of mucosal prostaglandin content, and diminishing capillary permeability.[9-11] Plant tannins are known to inhibit lipid peroxidation and to scavenge free radicals.[25] Tannins are known to 'tar' the outermost layer of the mucosa by their protein precipitating and vasoconstricting effects, and to render it less permeable and more resistant to gut secretions, and protect the underlying mucosa from toxins and other irritants.[26,27] This may prevent aggravation of ulcers. Therefore, we supposed that flavonoids and tannins in HP may help recover, or regress ulcers. Also, we thought that this herb contains a huge number of biologically active constituents; therefore, the mechanism of ulcer healing may not solely be attributed to the antioxidant properties of the herb. Several other mechanisms may be hypothesized as follows: 1. Hyperforin is one of the dual inhibitors of cyclooxygenase-1 and 5-lipoxygenase. Regarding the properties of dual cyclooxygenase-1 and 5-lipoxygenase inhibitors, hyperforin has anti-inflammatory activities devoid of gastric side effects seen for specific cyclooxygenase-1 and 5-lipoxygenase inhibitors.[28] Lipoxygenase products impair gastric mucosal integrity and exacerbate the damaging effects of noxious agents.[29] Recent reports showed that inhibition of leukotriene synthesis reduces gastric mucosal damage in different experimental models.[30] It could be that hyperforin has a contribution on antiulcerogenic activity of the herb by inhibition of cyclooxygenase-1 and 5-lipoxygenase. 2. Sutoo et al[31] reported that serum calcium is increased primarily by hypothermic stress, and increased serum calcium is transported to the brain where it may act to affect various central nervous system functions. Feißt and Werz[32] reported that hyperforin suppressed receptor mediated calcium mobilization by inhibition of G-protein signals. Glavin[33] reported that calcium channel antagonists reduced or abolished HRS induced gastric lesions. Therefore, we thought that hyperforin may suppress the effects of calcium enhanced by HRS.

In this study, our high dose produced less significant effects compared to the moderate dose. This could be explained, as mentioned by El-Sherbiny et al,[12] that low doses of HPE demonstrate antioxidant activity. In addition, high concentrations of HPE were reported to augment free radical levels, and low concentrations had antioxidant properties, and this effect is related to hypericin rather than hyperforin.[2] Abdel-Salam[13] reported that administration of HP inhibits gastric acid secretion in pyloric-ligated rats and exacerbated gastric lesions caused by indomethacin, however, he administered very high doses compared to ours. It was reported that using HP as an antidepressant might cause gastrointestinal irritations.[8] In this study, the resultant

effects of HPEs on ulcer indices may be hypothesized to due to the antioxidant properties of flavonoids, tannins, hypericin, and hyperforin, or inhibition of cyclooxygenase-1 and 5-lipoxygenase by hyperforin.

While this study was performed, the complexity of ulcer mechanism and pleiotropic effects of HP was not taken into account, as this study likewise, seek to reinvestigate the truth of traditional medicine practitioners on the usefulness of the aerial parts of HP for treatment of ulcers.

In conclusion, HPEs recover or regress gastric lesions induced by HRS in moderate doses. Further studies should be directed to determine the contributions of HP in gastric ulcer healing mechanisms as mentioned before.

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