Research Article / Araştırma Makalesi

The Potential Effect of Berberine on 5-Fluorouracil Induced Gastrointestinal Mucositis in Rats

Sıçanlarda 5-Fluorourasil ile Oluşturulmuş Gastrointestinal Mukozitte Berberinin Terapötik Etkisi

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Abstract: 5-Fluorouracil (5-FU), an antineoplastic agent, causes intestinal mucositis (IM) that limits the drug efficacy. The aim of this study was to reveal the protective and/or therapeutic effects of berberine (BER), an isoquinoline quaternary alkaloid, on IM induced by 5-FU in rats. Forty Wistar albino male rats were randomly divided into five groups: Control, 5-FU, 5-FU+BER75 (75 mg/kg/day), 5-FU+BER150 (150 mg/kg/day) and 5-FU+BER300 (300 mg/kg/day). 5-FU was intraperitoneally (i.p) injected at a dose of 60 mg/kg on alternate days for 4 days, while other groups were orally received BER daily for 7 days. By the end of study, intracardiac blood samples were collected and intestinal tissues were removed for histological examination. TNF-α, IL-6 and NF-κB levels were measured in serum using commercial ELISA kits. Results showed that BER at increasing doses ameliorated body weight loss and histopathological damage. It was concluded that BER may be effective in the treatment and/or prophylaxis of the damage in the gastrointestinal system caused by 5-FU in a dose-dependent manner.
Keywords: 5-Fluorouracil, Berberine, Gastrointestinal mucositis, In vivo

Özet: Çalışmamızda, sıçanlara 5-florourasil uygulanması ile oluşturulan gastrointestinal mukoza hasarına karşı berberinin terapötik potansiyelinin araştırılması. Gereç ve Yöntem: Çalışmada 6-8 haftalık Wistar Albino erkek sıçanlar kontrol, 5FU, BER75, BER150 ve BER300 olarak 5 gruba ayrılmıştır (n=8/grup). 5FU, BER75, BER150 ve BER300 gruplarına 0-7. günler arasında gün aşırı 5-FU (60 mg/kg, ip) uygulanarak mukozit oluşturuldu. BER75, BER150 ve BER300 gruplarına sırasıyla 75, 150 ve 300 mg/kg/gün dozlarda berberin yine 0-7. günler arasında hergün po uygulandı. Deneyin başında ve sonunda hayvanların vücut ağırlıkları ölçüldü. Total antioksidan aktivite (TAS), total oksidan aktivite (TOS), TNF-α, IL-6 ve NF-κB düzeylerinin serumda tayinleri için ELISA yöntemi kullanıldı. Jejenum ve ileum doku kesitlerinde histopatolojik değerlendirme yapıldı. Bulgular: Deneyin sonunda hayvanların vücut ağırlıklarındaki yüzde değişimi (%VA) tedavi gruplarında kontrol grubuna göre anlamlı derecede daha düşüktü (her biri için p <0,001), ancak berberin tedavisi 5-FU'in oluşturduğu kilo kaybını düzeltmemiştir. Oksidatif stres indeks değerleri açısından gruplar arasında anlamlı fark bulunamadı (p >0,05). IL-6 düzeyi 5FU grubuna kıyasla BER75 grubunda hafif, BER150 ve BER300 gruplarında anlamlı derecede azaldı (sırasıyla, p>0,05; p=0,002 ve p=0,002). TNF-α düzeyi 5-FU grubuna kıyasla BER75, BER150 ve BER300 gruplarında azaldı (sırasıyla p> 0,05; p=0,003; p> 0,05). NF-κB düzeyi kontrol grubuna kıyasla 5FU grubunda bir miktar yüksek bulunmuştur (p> 0,05). BER75, BER150 ve BER300 gruplarında ise anlamlı derecede azaldı (sırasıyla p<0.05, p<0,001 ve p<0.05). Histopatolojik olarak 5-FU grubunda yoğun epitel hasarı, inflamatuvar hücre infiltrasyonu, kanama ve ödem odakları görüldü. Tedavi gruplarında ise berberinin doz bağımlı bir şekilde bu hasarı azalttığı tespit edildi. Sonuç: Berberinin doz bağımlı olarak sıçanlarda 5-florourasil ile oluşturulan gastrointestinal mukozitte potansiyel bir terapötik ajan olabileceği düşünülmektedir.
Anahtar Kelimeler: 5-Florourasil, Berberin, gastrointestinal mukozit, invivo

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1. Introduction

5-fluorouracil (5-FU), an antimetabolite agent, has been used for almost 70 years in the treatment of several malignant tumors such as hepatocellular, breast, colorectal, head and neck cancer (1). It inhibits thymidylate synthase (TS) enzyme, which plays a role in nucleotide synthesis (2).

Because 5-FU has cytotoxic effects on tumor cells, it is also highly irritant and toxic drug for helathy cells. Therefore, one of the important challenges in the treatment with 5-FU is the narrowness of its therapeutic window (3). Although 5-FU has many hematological, dermatological, neurological, and acute cardiotoxic adverse effects (4-6), the most commonly seen adverse effects are in gastrointestinal system (nausea, vomiting, diarrhea and mucositis). One of the major adverse effects decreasing the treatment compliance of patients receiving 5-FU is mucositis which also reduces the quality of life (7).

Mucositis, can develop in entire gastrointestinal tract from the mouth to the anus, and increases the risk of ulceration and inflammation of the mucosal tissue. The pathological change in the intestinal mucosa caused by chemotherapy occurs in five stages. The formation and up-regulation of the chemotherapy-induced messenger signals, followed by the increase of mucosal damage, ulceration and the onset of the healing process via inflammatory mediators, respectively (7). The mechanisms involved in the development of mucositis due to 5-FU administration are oxidative stress, proinflammatory cytokines [especially IL-6, interleukin-1β (IL-1β), and TNF-alpha] and apoptosis (7, 8). Although opioid receptor agonists or cannabinoid receptor antagonists are currently used in the treatment of mucositis to inhibit the gastrointestinal motility, these treatments can also cause serious adverse effects (7).

It has been reported that essential oils, tannins, saponins, flavonoids and alkaloid derivatives play a role in the healing of mucositis by reducing the synthesis of proinflammatory cytokines (9). Berberine (BER), a constituent of Rhizoma coptidis (RC), commonly used for the treatment of gastroenteritis for a long time in Chinese medicine (10) is currently used as an over-the-counter drug for colitis, diarrhea, and gastroenteritis (11). In a previous study, BER was reported to improve 5-FU induced intestinal mucositis in mice by modulating the gut microbiota (12). There are also several studies reporting many beneficial effects of BER including antidiarrheic, anti-inflammatory, and antitumoral effects (13).

In our study, the preventive effect of BER in 5-FU-induced gastrointestinal damage in rats was evaluated through its anti-inflammatory activity. It is among the objectives of the study to enrich the literature on berberine and to be a step in bringing this active substance to modern medicine as well as traditional medicine.

2. Materials and Methods

Forty adult male Wistar Albino rats (8-10 weeks old, weighing 200-300 g) were used in the experiment. They were housed in a temperature (24±1°C) and relative humidity of 65%-70% controlled room with a 12:12-h light-dark cycle with food and water ad libitum.

Study design

5-FU (Kocak Farma, Turkey) has been reported to induce intestinal mucositis in rats by intraperitoneal (i.p.) administration for 7 days on alternate days (14). BER (Fluoro Chem, England) was administered daily for the same 7 days. Forty male Wistar albino rats were randomly divided into five groups (n=8/per group).

- Control group: Phosphate-buffered saline (PBS) (Biomatik, Canada) (p.o by gavage) for 7 days
- 5FU group: 5-FU (60 mg/kg) i.p for 7 days on alternate days
- BER75 group: Berberine (75 mg/kg, p.o, daily), 5-FU (60 mg/kg, i.p, on alternate days) for 7 days
- BER150 group: Berberine (150 mg/kg, p.o, daily), 5-FU (60 mg/kg, i.p, on alternate days) for 7 days

• BER300 group: Berberine (300 mg/kg, p.o, daily), 5-FU (60 mg/kg, i.p, on alternate days) for 7 days

At the end of the 7-day treatment period and 24 hours after the last drug dose, all animals were euthanized by cervical dislocation under high-dose general anesthesia. Intracardiac blood was collected, and centrifuged at 5000 rpm for 15 minutes at room temperature. Serum samples were stored at -80°C. The ileum and jejunum were removed and stored in 10% formaldehyde solution until histological evaluation. Animals were weighed on Day 0 (BW1) and on Day 7 (BW2) of the experiment. Body weight change (BW%) was calculated as percentages [BW% = (BW2–BW1)x100/BW1].

Biochemical evaluation

Commercially available IL-6 (Shanghai YI biotech Co. Ltd. Rat Interleukin 6 ELISA Kit, China), NF-kB (Shanghai YI biotech Co. Ltd. Rat NF-kB ELISA Kit, China) and TNF-α (Shanghai YI biotech Co. Ltd. Rat TNF-α ELISA Kit, China) ELISA kits were used to evaluate cytokine levels in rat plasma samples.

Histological evaluation

Jejunum and ileum tissues were fixed in 10% formaldehyde solution for 24 hours. After the fixed tissues were washed for 6 hours, they were turned into paraffin blocks by going through routine tissue follow-up processes (dehydration, clearing, paraffin impregnation and embedding in paraffin). 5 µm thick sections were taken from paraffin blocks and routine hematoxylin-eosin (H&E) staining (deparaffinization, hydration, HE dye steps and dehydration) was performed and the slides were closed with entellam. Images representing the findings of the examination performed under a binocular microscope were taken with a digital camera.

Statistical analysis

Statistical analysis was performed with SPSS Version 21.0 statistics software package program (IBM, Armonk, NY, USA). One-way analysis of variance (ANOVA) followed by post hoc Tukey's test was applied to the variables consisting of independent groups and showing normal distribution (IL-6). The comparison of continuous variables of more than two groups that did not meet the normal distribution was analyzed by the Kruskal-Wallis test (TNF-α, NF-κB). Descriptive data were expressed as mean± standard error of the mean (SEM) and median (25%-75% percentiles). The p-value less than 0.05 was considered as statistically significant.

3. Results

Body weight change

The percentage change in body weight (BW%) was significantly different in 5FU, BER75, BER150 and BER300 group compared to the control group ($p<0.001$). BW% was significantly decreased in 5-FU group compared to control group ($p<0.001$). Berberine treatment at the dose of 150 mg/kg slightly prevented weight loss induced by 5-FU ($p>0.05$) (Figure 1).

Biochemical evaluation

Serum IL-6 level was not found to be significantly different in 5-FU group compared to control group ($p>0.05$), however it was significantly decreased in the BER150 and BER300 groups compared to 5FU group ($p=0.002$) (Figure 2)

Serum TNF-α level was not found to be significantly different in 5-FU group compared to control group ($p>0.05$), however it was significantly decreased in the BER150 group compared to 5FU group ($p=0.003$). Serum NF-kB level was slightly increased in 5-Fu group compared to control group and it was significantly decreased in BER75, BER150 and BER300 groups compared to 5FU ($p<0.001$, $p=0.003$ and $p=0.043$, respectively) (Table 1)

Histological Evaluation

In contrast to the normal histological structure seen in both jejunum and ileum preparations of control group, epithelial damage, inflammatory cell infiltration, bleeding and edema foci were observed in the 5-FU group.

However, all these pathological changes seen in 5-FU group were more intense in the jejunum than the ileum. On the other hand, berberine treatment reduced the damage caused by 5-FU in a dose-dependent manner both in jejunum and ileum (Figure 3 and 4).

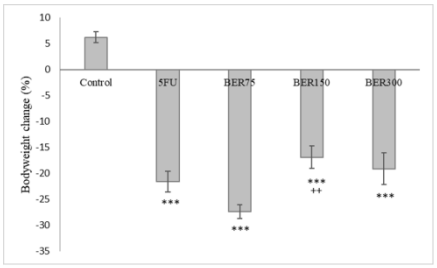

Figure 1. The percent change observed in body weight at the end of the study (BW%).

****, p<0.001 compared to control; ++, p<0.01 compared to BER75*

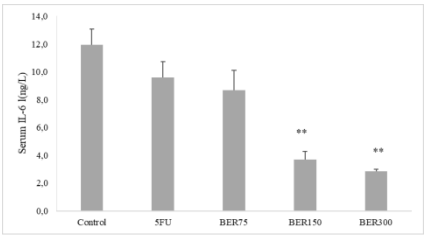

Figure 2. Serum IL-6 levels of rats in all groups.

***: p=0.002 compared to 5FU*
(One-way analysis of variance)

Table 1. Serum TNF-α and NF-κB levels

	Groups	N	Mean Std. error of mean	Median (25%-75%)	p	Pairwise comparisons
TNF-α (ng/l)	Control	8	109.843±10.820	92.06 (86.01-144.13)	p=0.003**	p=0.003 5FU vs BER150
	5FU	8	125.718±11.413	109.81 (104.98-148.47)		
	BER75	7	96.709±11.618	94.72 (77.51-118.73)		
	BER150	8	63.467±8.763	63.69 (46.34-81.98)		
	BER300	5	69.511±15.173	85.39 (43.79-96.44)		
NF-κB (ng/l)	Control	8	1.839±0.116	1.9635 (1.787-2.0375)	p=0.002**	p=0.003 BER150 vs 5FU; p=0.043 BER300 vs 5FU; p<0.001 BER75 vs 5FU
	5FU	8	2.262±0.113	2.281 (2.0235-2.49)		
	BER75	7	1.612±0.171	1.575 (1.3085-1.779)		
	BER150	8	1.402±0.086	1.3045 (1.2095-1.6315)		
	BER300	5	1.470±1.162	1.283 (1.2-1.823)		

Kruskal-Wallis One Way Analysis of Variance on Ranks (Median 25%-75%)

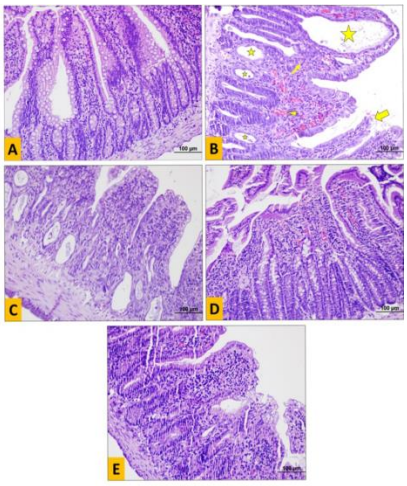

Figure 3. Hematoxylin-eosin staining of jejunum sections.

The control group (A) was found to have normal histology. In the 5FU group (B), epithelial damage (thick arrow), inflammatory cell infiltration (thin arrow), bleeding (arrowhead) and edema (star) foci were determined. It was determined that the damage caused by 5-FU was reduced in a dose-dependent manner in the groups administered 75 (C), 150 (D) and 300 (E) mg/kg berberine. All bars are 100 µm

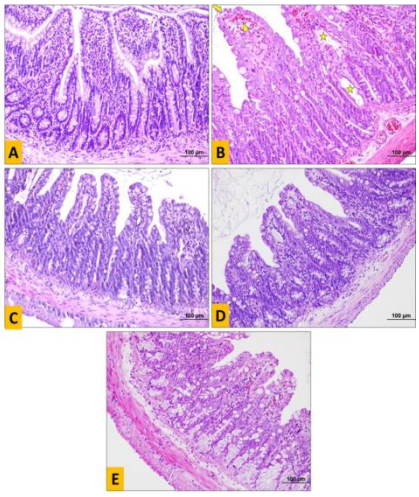

Figure 4. *Hematoxylin-eosin staining of ileum sections.*

The control group (A) was found to have normal histology. Epithelial damage (Bold arrow), bleeding (Arrowhead) and edema (Star) foci were determined in the 5FU group (B). It was determined that the damage caused by 5-FU was reduced in a dose-dependent manner in the groups administered 75 (C), 150 (D) and 300 (E) mg/kg berberine. All bars are 100 µm.

4. Discussion and Conclusion

This study was carried out to investigate the effect of berberine at different doses against

5-FU-induced tissue damage in the gastrointestinal tract in rats in terms of body weight change, cytokine levels and histological findings.

There are studies in the literature showing that there is a decrease in body weight of experimental animals due to 5-FU administration (15-17). Following the increase in apoptosis and decrease in proliferation in the intestines due to treatment with 5-FU, the homeostasis of crypt cells is impaired and the villus structures are shortened (12, 18). These processes result in the formation of intestinal mucositis. Worsening of intestinal mucositis with continued treatment results in decreased absorption of nutrients and loss of appetite and is the main cause of weight loss (19, 20). Moreover, some studies reported that death occurred in experimental animals due to excessive weight loss (20).

In the study of Chen et al (12), 100 mg/kg berberine treatment significantly decreased weight loss in rats treated with 40 mg/kg 5-FU. Although not stastitically different, weight loss was also slightly decresed in BER150 and BER300 groups in our study. Because it is possible that adverse effects of 5-FU can increase dose-dependently, higher 5-FU dose used in our study might resulted in the ineffectiveness of berberine to decrease the general toxic effects of 5-FU (21). In addition, the statistically significant higher weight loss in the BER75 group compared to the BER150 group can be considered as an insufficient berberine dose against intetinal mucositis induced by 5-FU (12). On the other hand, there was a slightly greater decrease in body weight in the BER300 group compared to the BER150, suggesting that the dose-dependent side effects of berberine may have an additional effect in 300 mg/kg berberine administration (22).

Two separate mechanisms play a fundamental role in the formation of intestinal mucositis. The first of these is the damage to cells, tissues and vessels following the formation of reactive oxygen derivatives due to chemotherapy. The other mechanism is through the synthesis of proinflammatory cytokines. The main cytokines synthesized are interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ). The synthesis of cytokines is the first step in the formation of advanced tissue damage (23, 24). All these processes are of great importance in the destructive effect of 5-FU on intestinal barrier functions (25).

It is known that one of the important processes that affect tissue damage with cytokines is the activation of nuclear factor kappa B (NF-κB). (25, 26). In their study, Pandey et al suggested that the effect of berberine is mediated by the NF-κB pathway, based on its regulatory effects on apoptotic, carcinogenic and inflammatory processes and on cyclooxygenase-2 (COX-2), IL-6, TNF-α levels (26). This study also supports our results that berberine decreased TNF-α and IL-6 proinflammatory cytokine levels besides the NF-κB level. Moreover, in a study conducted to prevent the development of mucositis induced by 5-FU through the inhibition of cytokines by using pentoxifylline and thalidomide, Lima et al. (27) reported that TNF-α inhibition has an important role in alleviating weight loss along with the improvement of diarrhea. In a similar study with cyclosporine, the effect of TNF-α inhibitor etanercept on chemotherapy-induced mucositis was investigated and it was reported that etanercept decreases the apoptosis induced in the intestinal epithelium, but does not cause any effect on diarrhea and weight loss (28). This can be explained by the possibility that cytokines other than TNF-α also have an important role in the development of chemotherapy-induced mucositis and gastrointestinal adverse effects (29). In this study, berberine treatment significantly decreased IL-6 and NF-κB levels, as well as TNF-α in varying doses, however, weight loss was less affected.

In accordance with the literature, histopathological evaluation in our study revealed normal jejunum and ileum tissues with no damage in the control group. On the other hand, tissue damage characterized by epithelial damage, inflammatory cell infiltration, hemorrhage and edema foci was observed in 5-FU group (17, 30, 31). It should

be noted that, 5-FU caused greatest damage on the jejunum compared to the ileum. All these histopathological changes were dose-dependently decreased in berberine-treated groups. Histopathological evaluation in this study support the biochemical and morphological findings and are compatible with the literature.

In summary, one of the most important results obtained from our study is the possibility that tissue damage in the gastrointestinal tract due to 5-FU administration can be prevented by increasing the dose of berberine. This study will contribute to the literature of berberine, which has been mentioned many times in different studies in the prevention of side effects that may occur in cancer chemotherapy

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Ethics

Ethics Committee Approval: The study was performed in accordance with the guidelines for the care and use of laboratory animals approved by the local Ethics Committee (Decision no:693-1, Date: 14.10.2022).
Informed Consent: The authors declared that it was not considered necessary to get consent from the patients because the study was a retrospective data analysis.
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