The Effectiveness of Grape Extract in Rats with Methotrexate Induced Intestinal Mucositis

Metotreksat ile İnce Bağırsak Mukoziti Oluşturulan Sıçanlarda Üzüm Ekstresinin Etkinliği

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Geliş Tarihi/*Received:* 12.12.2011
Kabul Tarihi/*Accepted:* 11.07.2012

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ABSTRACT Objective: To investigate histopathologically whether grape extract (Vitis Vinifera L), a potent free radical scavenger and can ameliorate the methotrexate -induced intestinal mucositis. **Material and Methods:** Wistar albino male rats were assigned to 3 groups: Group 1 (n=7) control group: An equal volume of distilled water that used for grape extract was given throughout the 15 days and physiological saline was administered intraperitoneally on the 10th day. Group 2 (n=10) methotrexate group: Rats received an equal volume of distilled water. A single dose of 20 mg/kg methotrexate was given intraperitoneally on the 10th day. Group 3 (n=10) methotrexate-grape extract group: The same dose of methotrexate was applied intraperitoneally on the 10th day. For milliliters grape extract solution was administered for 15 consecutive days. Complete blood counts were analyzed from each rat. **Results:** The control group showed normal intestinal structure. Group 2 had villus shortening and fusion with variable degrees of epithelial atrophy (p=0.001). The number of crypt cells was decreased and crypt loss was prominent (p=0.007). Inflammatory infiltration and decreased goblet cell number in both villi and crypts were distinctive (p<0.001). Villous and crypt injury, and inflammatory infiltration was less in group 3. In addition, goblet cells were increased. The difference was significant between group 2 and 3 for the jejunum damage (p=0.021). Hemoglobin levels increased in group 3. **Conclusion:** Present study suggested that grape extract could be useful in the ameliorating intestinal damage caused by methotrexate. Its effect could be attributed to its antioxidant properties. Grape extract may have a clinical application in cancer chemotherapy.

Key Words: Methotrexate; mucositis; grape seed extract; intestinal diseases

ÖZET Amaç: Potansiyel bir serbest radikal yok edici olan üzüm ekstresinin (Vitis Vinifera L) metotreksat ile oluşturulmuş ince bağırsak mukozitini histopatolojik olarak iyileştirip iyileştirmediğini araştırmak. **Gereç ve Yöntemler:** Wistar albino erkek sıçanlar 3 gruba ayrıldı. Grup 1 (n=7) kontrol grup: Üzüm ekstresi ile aynı miktarda olan distile su 15 gün boyunca kullanıldı ve 10. günde serum fizyolojik intraperitoneal olarak verildi. Grup 2 (n=10) metotreksat grubu: Sıçanlar aynı miktar distile su aldılar. Tek doz 20 mg/kg metotreksat 10. günde verildi. Grup 3 (n=10) Metotreksat-üzüm ekstresi grubu: Aynı doz metotreksat 10. günde intraperitoneal olarak uygulandı. Dört mililitre üzüm ekstresi solüsyonu 15 gün boyunca alındı. Tam kan sayımı her bir sıçandan analiz edildi. **Bulgular:** Kontrol grup normal ince bağırsak yapısını gösterdi. Grup 2 değişik derecelerde epitel atrofisi ile birlikte villüs kısalması ve füzyonuna sahipti (p=0.001). Kript hücrelerinin sayısı azalmış ve kript kaybı belirgindi (p=0.007). Hem villüs hem de kriptlerde inflamatuar infiltrasyon ve azalmış goblet hücreleri belirgindi (p<0.001). Grup 3'de villüs ve kript hasarı ile inflamatuar infiltrasyon azalmıştı. Aynı zamanda goblet hücreleri artmıştı. Grup 2 ve 3 arasındaki fark jejunum hasarı açısından istatistiksel olarak anlamlıydı (p=0.021). Üzüm ekstresi alan sıçanlarda hemoglobin değerleri artmıştı. **Sonuç:** Bu çalışma göstermiştir ki, üzüm ekstresi metotreksatın neden olduğu ince bağırsak hasarını iyileştirmede faydalı olabilmektedir. Etkisi antioksidan özelliğine atfedilebilir. Üzüm ekstresi kanser kemoterapisinde klinik uygulamaya sahip olabilir.

Anahtar Kelimeler: Metotreksat; mukozit; üzüm çekirdeği ekstresi; intestinal hastalıklar

doi: 10.5336/medsci.2011-27701

Turkiye Klinikleri J Med Sci 2012;32(6):1660-7

Mucositis is a histopathological formation of mucosal damage caused by chemotherapy.[1] Methotrexate (MTX) is an anticancer drug that inhibits the de novo synthesis of thymidine and purine which is resulted in the inhibition of proliferation of rapidly dividing cells such as cancer cells.[2] Since intestinal epithelial cells divide rapidly, MTX often causes mucosal inflammation of the intestine.[3] Intestinal epithelium injury is characterized by reduced mitoses in the crypts and shortened villi.[4] In the clinical using of this agent is often limited by its side effects such as severe nausea and widespread gastrointestinal ulceration resulted in diarrhea as a main clinical characteristic.

Reactive oxygen species (ROS) are responsible for the pathogenesis of the MTX-induced gastrointestinal mucosal damage.[5] Despite the exact mechanism of intestinal mucositis is unclear, no definitive prophylaxis or treatment were described.[6] Various treatment regimens have been tried to reduce MTX-induced intestinal damage.[7-15] However, these treatments have only been reported to partially prevent chemotherapy-induced intestinal mucositis. Effective treatment strategies are investigated.

Numerous experimental studies have now been exhibiting direct antioxidant activity of grape that protects cellular structures against peroxidation reactions owing to their rich phenolic contents. Grape seeds, grape skin and grape juice all are rich sources of phenolic compounds included catechin, epicatechin and dimeric, trimeric and tetrameric proanthocyanidins (PAs).[16] These molecules have an antioxidant property, which has been demonstrated to exert a novel spectrum of biological, pharmacological, therapeutic, and chemoprotective effects against oxygen free radicals and oxidative stress.[17-21] Resveratrol, one of the polyphenols identified in grape, also have chemopreventive and chemotherapeutic potential due to its antioxidant property.[22]

To better understand the action of dietary antioxidants on MTX-induced intestinal injury, present study was carried out to explore the protective effect of grape extracted from Kilis grape (*Vitis vinifera L.*) via histopathologic examination.

MATERIAL AND METHODS

ANIMALS

Wistar albino male rats were obtained from the Experimental Animal Research Center, Cukurova University Medical Faculty. The animals were kept in a temperature (21 ± 2 °C) and humidity (60 ± 5%) controlled room in which a 12–12 h light–dark cycle was maintained. Animals were fed a standard rat chow diet, had access to water ad libitum, and were synchronized by the maintenance of controlled environmental conditions (light, temperature, feeding time, etc.) The experiments were performed in accordance with the guidelines for Animal Research from the National Institute of Health and were approved by the Committee on Animal Research at Cukurova University, Turkey.

EXPERIMENTAL DESIGN

Wistar albino male rats, weighing 250-300 g, were assigned to 3 groups: Group 1 (n=7) control group: An equal volume of distilled water that used for grape extract was given with gavage tube throughout the 15 days and physiological saline (0.9% NaCl, in a similar dose of MTX) was administered intraperitoneally instead of MTX on the 10th day. Group 2 (n=10) MTX group: The rats received an equal volume of distilled water that was used for grape extract administration through the study. A single dose of 20 mg/kg MTX (500 mg in 20 ml vehicle, F.H. Faulding & Co. Ltd., Australia) was given intraperitoneally on the 10th day. Group 3 (n=10) MTX- grape extract (GE) group: The same MTX dose was administered intraperitoneally on the 10th day. GE solution was given for 15 consecutive days by a curved 3-in.-long 16-gauge gavage tube inserted after applying a proper restraint. Animals consumed an average of 4 mL of aqueous GE daily. On the 16th day all rats were sacrificed and three tissue samples of jejunum (0.5 cm) were cut off at a distance of 5 cm from the proximal end for histopathological evaluation. Blood samples were collected from each rat and complete blood counts were analyzed.

PREPARATION OF THE GRAPE EXTRACT

Red grapes known as Kilis Karası (Vitis vinifera L), the most popular winemaking grape cultivars, were

obtained from the Kilis region in the south of Turkey. The aqueous GE was prepared with using of 16 kg of grape. Undamaged and disease-free grapes snipped from their clusters were processed with seeds and skin in a domestic blender. The extract with skin, seed and juice was filtered and than boiled for evaporating the rest of their water. The extract was then stored in a dark bottle. The dilution of the concentrated extract was made in the proportion of 1:5 (extract/distilled water) for animal consumption. Elements of GE were analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) as follows: Fe: 18 mg/dL, Mg: 229 mg/dL, Ca: 365 mg/dL, Zn: 1.3 mg/dL. 3790 mg/L of polyphenol and 1.2 mg/L of resveratrol were measured by Gas Chromatography-Mass Spectrometry (GC-MS).

LIGHT MICROSCOPIC EXAMINATION

The tissue samples of the jejunum were fixed by 10% neutral formalin, embedded in paraffin and cut with a microtome set at 5 μm thickness. The tissue sections were stained with hematoxylin and eosin (HE) and examined under the light microscope. The sections were stained for mucin by the periodic acid-Schiff and Alcian blue (PAS-AB) technique at pH 2.5 to highlight the goblet cells. An overall score of tissue damage severity was semiquantitatively estimated as follows:[17] Villus damage, crypt damage, inflammatory cellular infiltration and goblet cell number. The histopathological appearance of each tissue was scored by a single observer (blinded to treatment) as the scores given to each criterion. Scores were given as no lesion = 0; mild damage = 1; moderate damage = 2, and severe damage = 3. The PAS-AB-stained goblet cells were counted under a x20 objective in both villi and crypts. For each specimen, a total of 10 microscopic fields of crypts and 10 fields of villi were measured per animal. Goblet cell numbers were scored as 0–49 cells = 3; 50–99 cells = 2; 100–149 cells = 1, and 150 cells = 0.

STATISTICAL ANALYSIS

The histopathologic scores were presented as the median (minimum-maximum). For the comparison of the histopathological results obtained from the three groups, Mann Whitney-U test was used; $p<0.05$ was considered to be statistically significant.

RESULTS

All rats in three groups had no significant changes in clinical observation such as body weight loss, diarrhea or bloody stools. The histopathological changes included villus damage, crypt damage, inflammatory cellular infiltration and goblet cell number in the jejunum tissue samples from the control and experimental groups are presented as the total score of intestinal damage in Table 1. Group 1 (control group) showed normal intestinal structure (Figure 1).

Group 2 (treated with MTX) compared to the control group: The jejunum tissue damage in MTX group was more severe than the control group (Figure 2). The tissue sections of the rats treated with MTX on day 16 had villus shortening and fusion with variable degrees of epithelial atrophy. The difference was significant statistically while comparing with MTX and control group ($p=0.001$). The light microscopic examination revealed that the number of crypt cells decreased and crypt loss was prominent. The difference between MTX and control group was statistically distinctive ($p=0.007$). Rats treated with MTX had increased leukocyte infiltration in the lamina propria and decreased goblet cell number in both villi and crypts compared to the rats that received saline solution. The difference was significant ($p<0.001$).

Group 3 (treated with MTX plus GE) compared to the rats treated with MTX group: Villus injury was less and the difference was significant statistically ($p<0.001$). Both the damage to crypts and inflammatory infiltration in the lamina propria continued to decrease in rats with treated MTX plus GE after 15 days (Table 1). There was a statistically significant difference between groups treated with MTX plus GE and only treated with MTX according to the cript damage ($p=0.002$) and cellular infiltration ($p=0.007$). Despite epithelial cell degeneration, an increased regeneration and goblet cell number were also depicted by light microscopic examination in rats given MTX plus GE.

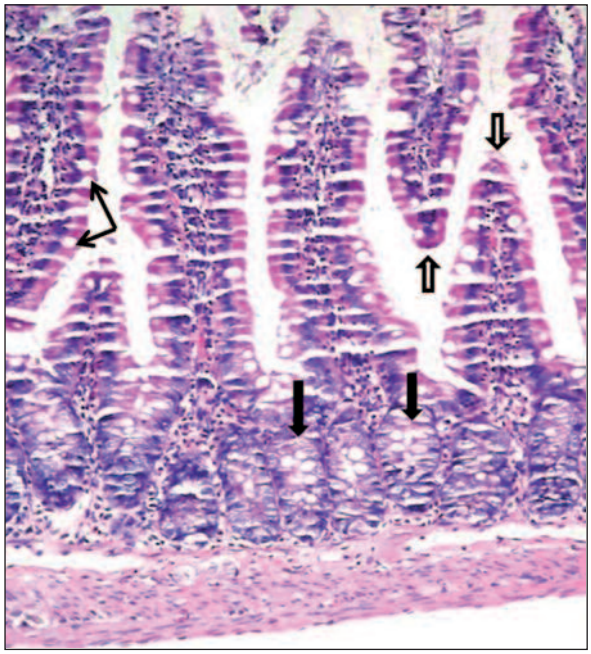

FIGURE 1: Control group: Jejunal morphology of rats on day 16. Villus (thick white arrows), crypt (thick black arrows), goblet cells (thin black arrows) are normal (HE, x200).
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A significant difference was found (p=0.011) (Figure 3).

In the evaluation of hematologic parameters, red blood cell counts and hemoglobin values were decreased in rats given only MTX (Table 2). Rats administered MTX plus GE presented high red blood cell counts (p=0.011) and hemoglobin (p=0.005) levels compared to rats only treated with MTX. Mean corpuscular volume (MCV) decreased in MTX plus GE group compared to group only treated with MTX. The difference was significant (p=0.005).

DISCUSSION

The use of chemotherapeutic drugs is limited due to their toxicity for normal proliferating cells, especially the rapidly-dividing cells of the intestinal crypt.[24] MTX has been shown to induce shortening or fusion of villi, and both atrophy and desquamation of the surface epithelium in various studies.[26-28] In the present study, the tissue sections of the rats showed normal intestinal structures in the saline-treated group. The barrier of intestinal mucosa integrity was changed and villus atrophy was remarkable in rats treated with MTX. Additionally, considerable morphological intestinal changes including crypt loss and polymorphonuclear leukocyte infiltration in the lamina propria were observed in the jejunum with the administration of only one single dose of MTX in our study. The MTX-induced damage to the small intestine has been attributed to the depressed generation of vil-

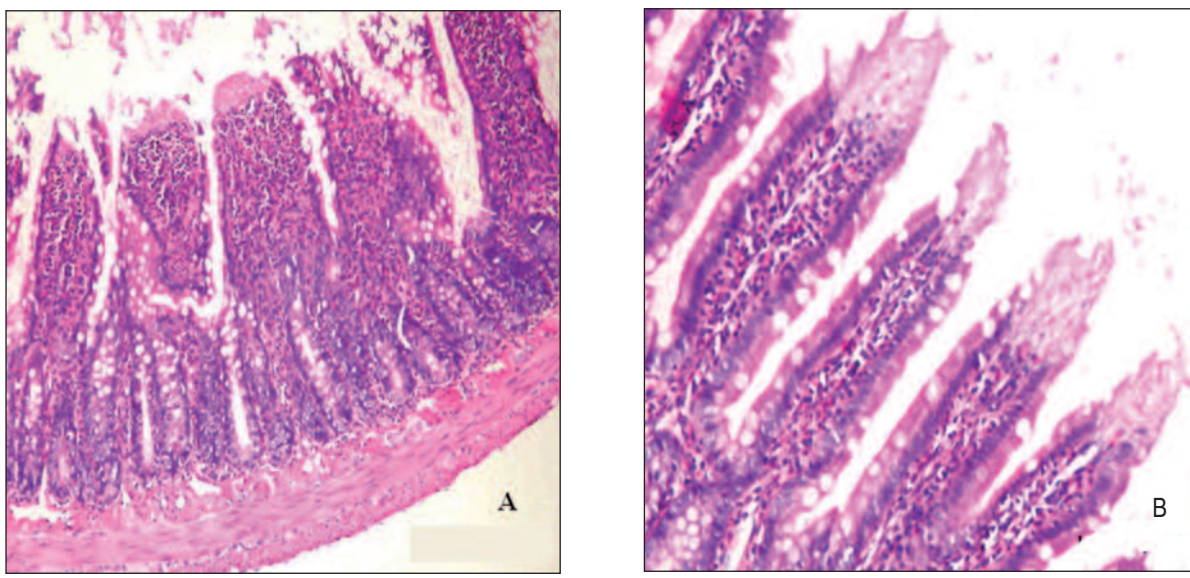

FIGURE 2: Methotrexate-treated group: Severe histopathological damage. A (HE, x100) and B (HE, x200). Villus shortening and fusion, crypt loss, inflammatory infiltration and goblet cell depletion.
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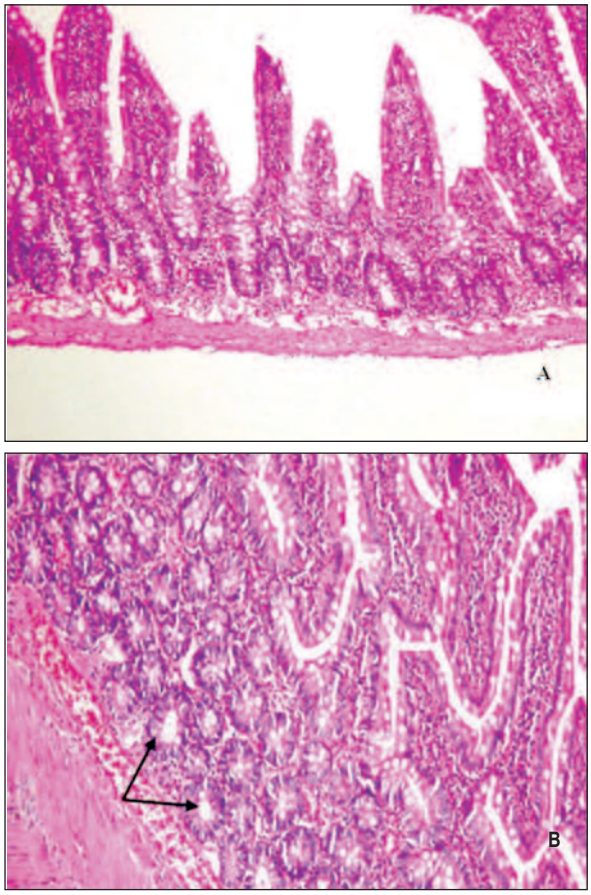

FIGURE 3: Methotrexate plus grape extract-treated group: A. The repairment of villus damage are seen (HE, x100). B. Increased crypt (black arrows) and goblet cells (HE, x200).
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lus epithelial cells, probably due to the damage of the crypt cells.[25] In the present study, the findings confirmed MTX-induced shortening and fusion of villi, epithelial atrophy and crypt loss. Additionally, other findings accompanied by polymorphonuclear leukocyte infiltration and goblet cell depletion were noticed by light microscopy in the damaged mucosa. Therefore injured intestinal mucosa had the same histopathological appearance in our rats treated with MTX, as reported in former investigations.

MTX treatment triggered a marked decrease of glutathione content as previously reported[7]. Glutathione depletion and resulting in oxidative stress also contribute to inflammatory pathway activation and intestinal inflammation enhancement. In order to minimize side-effects of MTX in patients, the studies focused on antioxidants.[28-31] One of them, GE, was mostly emphasized on its antioxidant properties in a number of in vitro and in vivo studies.[17,32,33] Various studies also presented that GE has been tried in the repair of MTX-induced intestinal injury. In the present study, the dilution of the concentrated GE which included skin, seed and juice was tried after inducing intestinal damage with MTX. One important finding of our study was that the reduced intensity of villi atrophy was observed by using of GE in histopathologic examination. Erlejman et al. demonstrated that proanthrocyanidines in GE were most effective in the preventing of oxidative damage in colonic cells.[34] It was stated that GE could prevent ulcerative damage in rat stomach.[35] We observed much crypt cell proliferation in MTX-GE treated rats compared to only MTX treated group. Although the exact mechanism of restoration of cell proliferative activity by GE remains unclear, it is likely

TABLE 1: Median (minimum-maximum) scores in the small intestine of rats on day 16 following the treatment with methotrexate alone and methotrexate plus grape extract. The severity of intestinal tissue damage in methotrexate plus grape extract-treated rats was less than that in methotrexate-treated rats.

Parameter Day 16	Control group n=7 median (minimum-maximum)	MTX-treated group n=10 median (minimum-maximum)	MTX+GE-treated group n=10 median (minimum-maximum)	Control group MTX-treated group p value	MTX-treated group/ MTX+GE-treated group p value
Villus damage	0.00 (0.00-1.00)	1.00 (1.00-3.00)	0.00 (0.00-1.00)	0.001	<0.001
Crypt damage	0.00 (0.00-0.00)	1.00 (0.00-3.00)	0.00 (0.00-0.00)	0.007	0.002
Cellular infiltration	0.00 (0.00-1.00)	2.00 (2.00-3.00)	1.00 (1.00-3.00)	<0.001	0.007
Goblet cell depletion	0.00 (0.00-0.00)	1.00 (1.00-2.00)	0.00 (0.00-2.00)	<0.001	0.011

MTX: Methotrexate; GE: Grape extract.

TABLE 2: Hematologic evaluation of study groups. Red blood cell count and hemoglobin values were higher in methotrexate plus grape extract group.

Variables	Control group n=7 median (minimum-maximum)	MTX-treated group n=10 median (minimum-maximum)	MTX+GE treated group n=10 median (minimum-maximum)	Control group/ MTX-treated group P value	MTX-treated group/ MTX+GE-treated group P value
WBC (cells/μL)	4400 (2800-11800)	5450 (1700-12900)	6300 (1190-11800)	0.883	1.000
LYM (cells/μL)	2200 (1600-7600)	3250 (1260-10200)	2345 (483-9440)	0.558	0.364
RBC (x106cells/μL)	8.06 (7.08-9.86)	5.65 (4.70-6.60)	6.85 (5.70-8.80)	0.001	0.011
HGB (g/100 mL)	14.0 (13.0-17.0)	14.7 (14.0-16.8)	17.1 (14.1-20.6)	0.525	0.005
HCT (%)	39.8 (36.0-49.6)	28.5 (24.3-35.2)	33.7 (27.9-43.0)	0.001	0.082
MCV (fL)	48.1 (46.5-50.8)	52.0 (48.3-53.4)	49.0 (46.9-51.5)	0.002	0.005
MCH (pg)	17.4 (17.1-19.2)	25.5 (21-30.0)	24.45 (19.6-34.0)	0.001	0.496
MCHC (g/dL)	36.4 (35.2-37.8)	50.1 (41.9-61.0)	50.0 (39.7-73.0)	0.001	0.970
RDW (%)	13.8 (13.3-15.4)	14.9 (13.7-17.1)	17.7 (15.2-22.9)	0.025	0.002
PLT (x10^3 cells/μL)	902000 (480000-1372000)	293500 (230000-420000)	337000 (50000-480000)	0.001	0.174

WBC: White blood cell count; LYM: Lymphocyte; RBC: Red blood cell count; HGB:Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell distribution width; PLT: Platelet; MTX: Methotrexate; GE: Grape extract.

that it is related to antioxidative effects of proantrocyanidines. It was shown that PAs increased glutathione peroxidase levels and decreased the jejunal damage caused by MTX treatment.[36] A similar result was proven by Yüncü et al. in an experimental study performed with the supplementation of vitamin A which had antioxidant properties.[37] In our animal model, the attenuating of villi damaged was noteworthy in the intestinal mucosa after the use of GE. Furthermore, increased crypt proliferation was noticeable in our study. It could be attributed that GE might have an effective role on oxidative damage and on antioxidant defense of intestinal mucosa which was exposed to MTX-induced oxidative stress. Taken together, previous published reports supported our current findings that GE treatment with MTX-administration led to significantly reversal of the shortening and fusion of villi and atrophy towards near normal.

Resveratrol, a polyphenol present in several plants including grapes, has been shown to have a number of physiological properties that could be useful in human medicine. A large range of resveratrol concentrations (0.05 to 10 mg/L) was found in grape juices.[38] We measured the value of resveratrol as 1.2 mg/L within the GE. Extensive study over the past decade has shown both the chemopreventive and chemotherapeutic potentials of resveratrol.[22,39] In addition it was shown that resveratrol might prevent the death of erythrocytes which means eryptosis.[40] Rodrigue et al. reported that resveratrol induced a higher hemoglobin production in cell line.[41] In the present experimental study, hemoglobin level increased in MTX-GE treated rats compared to MTX treated group. Therefore, GE could have an advantage in increasing the hemoglobin level in clinical practice.

Small intestine injuries may contribute to increased morbidity and mortality in cancer patients and limitation of an effective chemotherapy treatment. Rapidly proliferative cells of the gastrointestinal system are vulnarable to the methotrexate toxicity.[24] In this context, we thought that naturally occurring dietary supplements could be used in combination with chemotherapy to ameliorate the mucosal damage and stimulate the tissue repairing of intestine. The major objectives of the current study were to assess the preventive effect of GE histopathologically on MTX-induced villus damage, epithelial atrophy, crypt loss, neutrophil accumulation in the lamina propria and goblet cell depletion in the small intestine of rats.

CONCLUSION

The histopathological changes of present experimental study confirmed that the intake of whole GE should be recommended in dietary habits as a potential source of antioxidant supplement to prevent or limit intestinal mucositis caused by MTX. Our results showed that GE should be tried to decrease the MTX-induced intestinal damage in several days prior to and after treatment with MTX. The regular consumption of a GE could positively modulate the treatment of intestinal mucositis and hemoglobin level in clinical settings. GE not only could have a beneficial role in the ameliorating of intestinal mucositis during cancer chemotherapy, but also might be a therapeutic bridge in case of allowing time for other therapies to become effective in cancer patients. However, further in vivo studies should be performed to identify the exact biological mechanism and antioxidant properties of GE in human body.

REFERENCES

1. Özkalemkaş F. [Mucositis.] Turkiye Klinikleri J Hem Onc-Special Topics 2011;4(2):50 -6.
2. Ortiz Z, Shea B, Suarez-Almazor ME, Moher D, Wells GA, Tugwell P. The efficacy of folic acid and folinic acid in reducing methotrexate gastrointestinal toxicity in rheumatoid arthritis. A metaanalysis of randomized controlled trials. J Rheumatol 1998;25(1):36-43.
3. Taminiau JA, Gall DG, Hamilton JR. Response of the rat small-intestine epithelium to methotrexate. Gut 1980;21(6):486-92.
4. Altmann GG. Changes in the mucosa of the small intestine following methotrexate administration or abdominal x-irradiation. Am J Anat 1974;140(2):263-79.
5. Jahovic N, Cevik H, Sehirli AO, Yeğen BC, Sener G. Melatonin prevents methotrexate-induced hepatorenal oxidative injury in rats. J Pineal Res 2003;34(4):282-7.
6. Symonds RP. Treatment-induced mucositis: an old problem with new remedies. Br J Cancer 1998;77(10):1689-95.
7. Jahovic N, Sener G, Cevik H, Ersoy Y, Arbak S, Yegen BC. Amelioration of methotrexate-induced enteritis by melatonin in rats. Cell Biochem Funct 2004;22(3): 169-78.
8. surui K, Kosakai Y, Horie T, Awazu S. Vitamin A protects the small intestine from methotrexate-induced damage in rats. J Pharmacol Exp Ther 1990;253(3):1278-84.
9. Horie T, Nakamaru M, Masubuchi Y. Docosahexaenoic acid exhibits a potent protection of small intestine from methotrexate-induced damage in mice. Life Sci 1998;62(15):1333-8.
10. Horie T, Matsumoto H, Kasagi M, Sugiyama A, Kikuchi M, Karasawa C, et al. Protective effect of aged garlic extract on the small intestinal damage of rats induced by methotrexate administration. Planta Med 1999;65(6):545-8.
11. Gao F, Nakamaru M, Masubuchi Y, Horie T. Protective effect of a synthetic analog of prostaglandin E(1) on the small intestinal damage induced by the administration of methotrexate to rats. J Pharm Sci 2001;90(8):1040-8.
12. Miyazono Y, Gao F, Horie T. Oxidative stress contributes to methotrexate-induced small intestinal toxicity in rats. Scand J Gastroenterol 2004;39(11):1119-27.
13. Gibson RJ, Keefe DM, Clarke JM, Regester GO, Thompson FM, Goland GJ, et al. The effect of keratinocyte growth factor on tumour growth and small intestinal mucositis after chemotherapy in the rat with breast cancer. Cancer Chemother Pharmacol 2002;50(1):53-8.
14. Howarth GS, Cool JC, Bourne AJ, Ballard FJ, Read LC. Insulin-like growth factor-I (IGF-I) stimulates regrowth of the damaged intestine in rats, when administered following, but not concurrent with, methotrexate. Growth Factors 1998;15(4):279-92.
15. Gao F, Horie T. A synthetic analog of prostaglandin E(1) prevents the production of reactive oxygen species in the intestinal mucosa of methotrexate-treated rats. Life Sci 2002;71(9):1091-9.
16. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, et al. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology 2000;148(2-3):187-97.
17. Cetin A, Kaynar L, Koçyiğit I, Hacioğlu SK, Saraymen R, Oztürk A, et al. The effect of grape seed extract on radiation-induced oxidative stress in the rat liver. Turk J Gastroenterol 2008;19(2):92-8.
18. Lutz M, Jorquera K, Cancino B, Ruby R, Henriquez C. Phenolics and antioxidant capacity of table grape (Vitis vinifera L.) cultivars grown in Chile. J Food Sci 2011;76(7):C1088-93.
19. Mansouri E, Panahi M, Ghaffari MA, Ghorbani A. Effects of grape seed proanthocyanidin extract on oxidative stress induced by diabetes in rat kidney. Iran Biomed J 2011;15(3):100-6.
20. Lionetto MG, Giordano ME, Calisi A, Erroi E, De Nuccio F, Schettino T. Effect of the daily ingestion of a purified anthocyanin extract from grape skin on rat serum antioxidant capacity. Physiol Res 2011;60(4):637-45.
21. Bagchi D, Garg A, Krohn RL, Bagchi M, Tran MX, Stohs SJ. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro. Res Commun Mol Pathol Pharmacol 1997;95(2):179-89.
22. Kundu JK, Surh YJ. Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. Cancer Lett 2008;269(2):243-61.
23. Vardi N, Parlakpinar H, Ozturk F, Ates B, Gul M, Cetin A, et al. Potent protective effect of apricot and beta-carotene on methotrexate-induced intestinal oxidative damage in rats. Food Chem Toxicol 2008;46(9):3015-22.
24. Leblond J, Le Pessot F, Hubert-Buron A, Duclos C, Vuichoud J, Faure M, et al. Chemotherapy-induced mucositis is associated with changes in proteolytic pathways. Exp Biol Med (Maywood) 2008;233(2):219-28.
25. Yáñez JA, Teng XW, Roupe KA, Fariss MW, Davies NM. Chemotherapy induced gastrointestinal toxicity in rats: involvement of mitochondrial DNA, gastrointestinal permeability and cyclooxygenase -2. J Pharm Pharm Sci 2003;6(3):308-14.
26. Taminiau JA, Gall DG, Hamilton JR. Response of the rat small-intestine epithelium to methotrexate. Gut 1980;21(6):486-92.
27. Naruhashi K, Nadai M, Nakao M, Suzuki N, Nabeshima T, Hasegawa T. Changes in absorptive function of rat intestine injured by methotrexate. Clin Exp Pharmacol Physiol 2000;27(12):980-6.

28. Pinkerton CR, Milla PJ. Methotrexate enterotoxicity: influence of drug dose and timing in the rat. Br J Cancer 1984;49(1):97-101.
29. Maeda T, Miyazono Y, Ito K, Hamada K, Sekine S, Horie T. Oxidative stress and enhanced paracellular permeability in the small intestine of methotrexate-treated rats. Cancer Chemother Pharmacol 2010;65(6):1117-23.
30. Sener G, Ekşioğlu-Demiralp E, Cetiner M, Ercan F, Yeğen BC. Beta-glucan ameliorates methotrexate-induced oxidative organ injury via its antioxidant and immunomodulatory effects. Eur J Pharmacol 2006;542(1-3):170-8.
31. Kesik V, Uysal B, Kurt B, Kismet E, Koseoglu V. Ozone ameliorates methotrexate-induced intestinal injury in rats. Cancer Biol Ther 2009;8(17):1623-8.
32. Peluzio Mdo C, Teixeira TF, Oliveira VP, Sabarense CM, Dias CM, Abranches MV, et al. Grape extract and α-Tocopherol effect in cardiovascular disease model of Apo E -/- Mice. Acta Cir Bras 2011;26(4):253-60.
33. Pinheiro FV, Pimentel VC, De Bona KS, Scola G, Salvador M, Funchal C, et al. Decrease of adenosine deaminase activity and increase of the lipid peroxidation after acute methotrexate treatment in young rats: protective effects of grape seed extract. Cell Biochem Funct 2010;28(1):89-94.
34. Erlejman AG, Fraga CG, Oteiza PI. Procyanidins protect Caco-2 cells from bile acid- and oxidant-induced damage. Free Radic Biol Med 2006;41(8):1247-56.
35. Saito M, Hosoyama H, Ariga T, Kataoka S, Yamaji N. Antiulcer activity of grape seed extract and procyanidins. J Agric Food Chem 1998;46(4):1460-4.
36. Gulgun M, Erdem O, Oztas E, Kesik V, Balamtekin N, Vurucu S, et al. Proanthocyanidin prevents methotrexate-induced intestinal damage and oxidative stress. Exp Toxicol Pathol 2010;62(2):109-15.
37. Yüncü M, Eralp A, Koruk M, Sari I, Bağci C, Inalöz S. Effect of vitamin A against methotrexate-induced damage to the small intestine in rats. Med Princ Pract 2004;13(6):346-52.
38. Goldberg DM, Tsang E, Karumanchiri A, Diamandis E, Soleas G, Ng E. Method to assay the concentrations of phenolic constituents of biological interest in wines. Anal Chem 1996;68(10):1688-94.
39. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Res 2004;24(5A):2783-840.
40. Qadri SM, Föller M, Lang F. Inhibition of suicidal erythrocyte death by resveratrol. Life Sci 2009;85(1-2):33-8.
41. Rodrigue CM, Arous N, Bachir D, Smith-Ravin J, Romeo PH, Galacteros F, et al. Resveratrol, a natural dietary phytoalexin, possesses similar properties to hydroxyurea towards erythroid differentiation. Br J Haematol 2001;113(2):500-7.