Biotechnic & Histochemistry

ISSN: 1052-0295 (Print) 1473-7760 (Online) Journal homepage: https://www.tandfonline.com/loi/ibih20

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**To cite this article:** S. Gedik, ME Erdemli, M Gul, B Yigitcan, H Gozukara Bag, Z Aksungur & E Altinoz (2018) Investigation of the protective effects of crocin on acrylamide induced small and large intestine damage in rats, Biotechnic & Histochemistry, 93:4, 267-276, DOI: 10.1080/10520295.2018.1432888

**To link to this article:** https://doi.org/10.1080/10520295.2018.1432888

Published online: 12 Apr 2018.

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# Investigation of the protective effects of crocin on acrylamide induced small and large intestine damage in rats

S. Gedik[1], ME Erdemli[2], M Gul[3], B Yigitcan[3], H Gozukara Bag[4], Z Aksungur[5], E Altinoz[6]

[1]*Department of Chemistry, Faculty of Sciences, Karabuk University, Karabuk,* [2]*Department of Medical Biochemistry, Medical Faculty, Nigde Omer Halisdemir University, Nigde,* [3]*Department of Histology and Embryology, Medical Faculty, Inonu University, Malatya,* [4]*Department of Biostatistics, Medical Faculty, Inonu University, Malatya,* [5]*Department of Medical Biochemistry, Medical Faculty, Inonu University, Malatya, and* [6]*Department of Medical Biochemistry, Medical Faculty, Karabuk University, Karabuk, Turkey*

## Abstract

We investigated repair of acrylamide (AA) induced damage in intestines by administration of crocin. We used 40 male Wistar rats in four groups of 10 animals: control, AA, crocin, and AA + crocin groups. We investigated biochemical and histological changes to small and large intestine. AA ingestion decreased glutathione (GSH) levels and total antioxidant status (TAS) in the intestine compared to the control group, while superoxide dismutase (SOD) and catalase (CAT) activities, and total oxidant status (TOS) and malondialdehyde (MDA) levels were increased. Villi were shortened and villus degeneration was observed in ileum of the AA group. Degeneration of surface epithelium and Liberkühn crypts were observed in colon sections. GSH and TAS levels increased after administration of AA together with crocin, while SOD and CAT levels and TOS and MDA levels decreased; significant recovery of histological damage also was observed. We found that crocin exhibits protective effects on AA induced small and large intestine damage by inhibiting oxidative stress.

**Key words**: acrylamide, crocin, large intestine, oxidative stress, rats, small intestine

Acrylamide (AA) was synthesized initially for use by American industries during the 1950s. AA is the monomer used for polyacrylamide synthesis for industrial purposes including cosmetic products and paper manufacturing. Polyacrylamides are chemically inert, nontoxic and generally stable structures. Polyacrylamide is used for treatment of potable water (Sharp 2003, LoPachin and Canady 2004) and industrial waste water, construction of potable water reservoirs and sewer systems (Rudén 2004), the plastics industry (Shaw and Thomson 2003), gel electrophoresis (Barber et al. 2001), the paint industry and mining (Konings et al. 2003).

AA exhibits toxic effects and can cause cancer. It has been reported that AA is formed in foods owing to heat treatment (Tareke et al. 2000, Tareke et al. 2002) and this has increased interest in the effects of AA. At temperatures ≥ 120° C, the reaction of glucose with the amino acid, asparagine, in carbohydrates and protein-rich foods, produces AA (Stadler et al. 2002, Mottram et al. 2002). Although the process potentially threatens human health, consumption of AA continues, because most humans desire a carbohydrate and protein based diet. The United States Environmental Protection Agency (EPA) published reference values for normal and toxic AA levels. Lifetime risk for cancer is 0.7 – 4.5/1,000 based on consumption of 1 µg AA/kg body weight/day for humans (EPA 1985). WHO guidelines set the acceptable AA level in potable water at <0.5 µg/l (S'witzerland 1993), while the European Union determined the level to be < 0.1 µg/l

Correspondence: E Altinoz, Department of Medical Biochemistry, Medical Faculty, Karabuk University, Karabuk, Turkey. E-mail: eyupaltinoz@karabuk.edu.tr

*Biotechnic & Histochemistry* 2018, **93(4):** 267–276

(European Council Directive 1998). According to acute toxicity studies, the oral LD50 for AA is approximately 100 – 150 mg/kg in mice, rabbits, guinea pigs and rats (Hashimoto et al. 1981, Paulet 1975).

AA is absorbed in the gastrointestinal tract and transported to the liver by hemoglobin (4 mol AA/mol hemoglobin) (Kopp et al. 2008). AA is metabolized in the body by two pathways. The first pathway is conversion of AA to glycidamide, a genotoxic and mutagenic substance that is formed in the liver by CYP2E1, a cytochrome P450 (CYP) dependent isoenzyme (Arinç et al. 2007). CYP enzyme systems are found in both the liver and mucosal epithelium of the digestive tract (Shimizu et al. 1990). CYP2E1 plays an important role in the metabolism of both external xenobiotics, such as AA, and internal compounds (Nelson et al. 1993), and could initiate lipid oxidation, which produces reactive oxygen species (ROS) (Lieber 1997). CYP2E1 appears to participate in the pathogenesis of tissue injury by producing harmful intermediary products, such as ROS and lipid peroxides (Lee et al. 1995, Dai et al. 1993). CYP3A is a subfamily that is expressed in the liver and intestinal tissues that oxidizes xenobiotics and various endogenous compounds, and plays an important role in inactivation of CYP3A xenobiotics. AA, which is converted to glycidamide by CYP2E1 in the liver, exhibits mutagenic effects and causes cancers in the lung, brain, kidney, uterus and testis in experimental animals (Arinç et al. 2007).

The second pathway is conjugation of AA with reduced glutathione (GSH) by glutathione-S-transferase (GST), which uses GSH as a coenzyme and converts it to nontoxic N-acetyl-S-(2-carbomoylethyl) cysteine followed by excretion in urine (Sumner et al. 1999, Ghanayem et al. 2000). CYP2E1 and GST enzymes compete for AA as a substrate (Calleman et al. 1990, Sumner 1992). As oral AA intake increases, levels of GSH in tissues may decrease, which favors formation of oxidants. AA cannot be detoxified by GST owing to decreased GSH levels; it is metabolized to the more toxic glycidamide by the CYP2E1 enzyme system (Sumner et al. 1999). Glycidamide is transported to tissues by hemoglobin and interacts with cellular DNA, which leads to the formation of cancer cells (Besaratinia and Pfeifer 2004).

The body possesses endogenous antioxidant enzymes that constitute an effective defense system to neutralize and protect the damage caused by free radicals (Ak et al. 2005). It has been proposed that the oxidative damage caused by cigarette smoking, alcohol consumption and other xenobiotic substances also could apply to AA or influence the metabolism of AA to glycidamide (Moldoveanu and Gerardi 2011, Vikstrom et al. 2010). Oxidative stress is caused by disequilibrium between antioxidants and oxidants in favor of oxidants, which permits ROS to attack macromolecules such as DNA, lipids and proteins (Naruszewicz et al. 2009). ROS play a key role in pathogenesis of many diseases including aging, cardiovascular diseases, cancer, renal diseases, neurological diseases, and muscle and liver diseases (Freeman and Crapo 1982).

Toxins, radiation, cigarette smoking, alcohol consumption, pathogenic substances, intense and irregular lifestyle, intensive exercise and weak antioxidant defense system can cause oxidative stress. Under these circumstances, antioxidants are activated and interact with free radicals to prevent cellular damage. Endogenous enzymatic antioxidant systems include catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), as well as non-enzymatic systems including uric acid, bilirubin and glutathione (GSH). SOD and CAT play an important role in protecting against the adverse effects of lipid peroxide and hydrogen peroxide ($H_2O_2$) (Zhu et al. 2012). GSH, a non-enzymatic antioxidant that participates in physiological antioxidant defense, could neutralize free radicals, stabilize sulfhydryl groups and reduce hydrogen peroxide (He et al. 2012). Various antioxidant compounds including vitamin E (tocopherol), vitamin C (ascorbic acid), beta-carotene, flavonoids, saffron, coenzyme Q, lycopene, etc. also may be consumed to help combat elevated ROS.

Saffron (*Crocus sativus L.*) is an iridaceous plant that is indigenous to Iran, Spain, Kashmir (India and Pakistan), Greece, Azerbaijan, China, Morocco, Mexico, Libya, Turkey and Austria. In addition to its antioxidant properties, saffron is used in traditional medicine for its hypolipidemic, anti-inflammatory and anti-carcinogenic properties (Rios et al. 1996). Crocin (crocetin glycoside), crocetin and safranal are the main active ingredients in saffron. Saffron contains proteins, sugars, vitamins, flavonoids, amino acids and minerals (Bathaie and Mousavi 2010). Saffron commonly is used as a medicinal supplement and as a traditional medicine for treating illnesses including depression, mental disorders and cancer (Schmidt et al. 2007).

We investigated damage to the small and large intestines of rats exposed to ingested AA and the therapeutic effect of crocin owing to its free radical scavenging antioxidant properties.

## Material and methods

### Animals

We used 40 225 – 250 g male Wistar albino rats obtained from Inonu University, Faculty of Medicine, Experimental Animal Breeding and Research Center (INUTF-DEHUM). Our study was approved by the experimental animal ethics committee (2015/A-97). The rats were housed at 21° C, 55 – 60% ambient humidity under 12 h light (08:00 to 20:00):12 h dark conditions. Rats were fed *ad libitum* with standard pellet feed throughout the study. Drinking water was changed and the cages were cleaned daily.

### Experimental design

Rats were divided randomly into four groups of 10: control (C) group, given 1 ml physiological saline; crocin (Cr) group, given 50 mg/kg crocin (42,553–65-1; Sigma Aldrich, St. Louis, MO) (Hariri et al. 2010); AA (AA) group given 25 mg/kg AA (Sigma Aldrich) (Burek et al. 1980); and AA + crocin (AA + Cr) group, given 25 mg/kg AA together with 50 mg/kg crocin. All administered chemicals were dissolved in saline and each rat was given 1 ml/kg/day by gavage. All applications were at the same time of day every day for 21 days.

### Samples

At the end of the experiment, the rats were decapitated under xylazine-ketamine anesthesia and approximately 2 cm samples of ileum and colon tissue were obtained. After the intestinal content was drained, the tissues were cleaned with physiological saline. A small block of each tissue was fixed in 10% formaldehyde for histopathological examination and the remaining portion was stored at –80° C for biochemical analyses.

### Biochemistry

On the day of analysis, tissues were removed from the freezer and weighed. Phosphate buffer was added and the tissues were homogenized at 12,000 rpm for 1 – 2 min on ice to create a 10% homogenate (IKAUltra Turrax T 25 basic, IKA Labotechnik, Staufen, Germany). Malondialdehyde (MDA) levels were measured in the homogenate. The remaining homogenate was centrifuged at 600 x g at 4° C for 30 min to obtain the supernatant. GSH, SOD, CAT, total antioxidant status (TAS), total oxidant status (TOS) and protein levels were measured in the supernatant.

MDA analysis was conducted according to the method described by Ohkawa et al. (1979). Tissue homogenate, 0.5 ml, was mixed with 3 ml 1% $H_3PO_4$ and 1 ml 0.6% thiobarbituric acid. The mixture was heated in a boiling water bath for 45 min and extracted in 4 ml n-butanol; n-butanol was used as a blank and tetramethoxypropane was used as a standard. MDA levels were measured at 535 nm using a spectrophotometer (T80 UV/VIS Spectrometer, PG Instruments Ltd., Leicestershire, UK).

GSH was measured using the method described by Ellman (1959). A yellow-green color forms as a result of the reaction between the glutathione in the medium after addition of 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) to the flasks. The amount of reduced glutathione was determined by measuring the intensity of the resulting color at 410 nm using a spectrophotometer.

SOD activity was measured using the method reported by Sun et al. (1988). Superoxide radicals are produced by xanthine-xanthine oxidase. The superoxide radical creates a color by reducing NBT (nitro blue tetrazolium) to a blue-colored formazan. The absorbance at 560 nm of the formazan produced is used to calculate the SOD activity. Distilled water was used as a blank. SOD activity was expressed as U/g protein.

CAT activity was measured using the method reported by Aebi and Bergmeyer (1974). Hydrogen peroxide ($H_2O_2$) absorbs ultraviolet light; the wavelength of maximum absorption is 240 nm. The decomposition of $H_2O_2$ into water and oxygen by catalase in the supernatant is measured by reduction of absorbance at 240 nm. The observed decrease in absorbance was recorded for 1 min to measure the activity of the enzyme.

Tissue TOS levels were measured according to Erel (2005). A total oxidant status kit (Rel Assay Diagnostics, Gaziantep Turkey) was adapted to the Biotek biochemical auto-analyzer (BioTek Instruments, Inc., Winooski, VT). The oxidants in the sample convert ferrous ion chelator complexes to ferric ions. The ferric ions form a colored complex with the chromogenic solution. This complex was measured spectrophotometrically at 530 nm and is directly proportional to the amount of

oxidant in the sample. The results are expressed as µmol $H_2O_2$ equiv/l.

TAS levels were measured according to Erel (2004). TAS was measured using a Rel Assay brand kit (Rel Assay Diagnostics, Gaziantep Turkey). The measurement is based on decolorization of antioxidant molecules. We combined 500 µl reagent 1 (measurement buffer) and 30 µl supernatant according to the kit instructions and the absorbance was measured at 660 nm with an ELISA adjusted to 25° C to determine the TAS levels. Then, 75 µl reagent 2 (colored ABTS solution) was added to the mixture and the product was incubated for 10 min. TAS levels were determined by reading the absorbance at 660 nm after incubation. Trolox, a water soluble analog of vitamin E, was used as a calibrator. The results are expressed as mmol trolox equiv/l.

### Histology

The ileum and colon samples were fixed for 48 h in 10% neutral buffered formalin at room temperature. Intestine specimens were dehydrated through 50, 70, 80, 96, and 99.9% ethanols, cleared in xylene and embedded in paraffin. Paraffin embedded specimens were cut at 6 µm, mounted on slides and stained with hematoxylin and eosin (H & E) (Bancroft and Gamble 2002). The degree of histopathologic damage to the ileum (villous stunting, epithelial injury, villous injury, Liberkühn crypt injury and inflamation) and colon (goblet cell depletion, epithelial injury, mucosal fibrosis, Liberkühn crypt injury and inflammation) were assessed on each slide and scored as: 0, normal histology; 1, mild damage; 2, moderate damage; 3, severe damage for a maximum score of 15 for ileum and colon sections. Sections were examined by an histologist, blinded to the identity of the specimen, using a Nikon Optiphot-2 light microscope and camera (Nikon DS-Fi 2) and analyzed using an Image Analysis System (DS L3; Nikon Corp., Tokyo, Japan) (Erben et al. 2014).

### Statistical analysis

SPSS 21 software was used for statistical analysis. Means ± SD were calculated. Normal distribution of data was examined by Shapiro Wilk test. The Levene test was used to determine the homogeneity of the variances. One way ANOVA followed by Tukey HSD paired comparison was used to compare groups with homogeneous variances. In cases of nonhomogeneous variances, the Welch test and Tamhane T2 paired comparison method were used. Histological scoring was ordinal, so we used nonparametric tests for group comparisons. Because nonparametric tests use ranking rather than actual values and comparisons are not based on mean values, we used median values as a measure of central tendency. For histological scoring, data were summarized using median, minimum and maximum values. The Kruskal-Wallis test was used to compare the groups and the Conover method was used for paired comparisons. The significance level was set as $p \leq 0.05$ for all tests.

## Results

### Biochemistry

AA administration alone caused significantly increased MDA levels in the small and large intestine compared to controls ($p < 0.05$); however, GSH levels decreased significantly compared to controls ($p < 0.05$). MDA levels were decreased in the small intestine by crocin treatment in the AA + Cr group ($p < 0.05$) compared to the AA group, while a significant increase was observed in GSH levels ($p < 0.05$) compared to the AA group. MDA and GSH levels improved after crocin treatment in large intestine of the AA + Cr group compared to the AA group, but the changes were not statistically significant.

SOD and CAT activities increased significantly in small and large intestines in the AA group compared to the C group ($p < 0.05$), while significant decreases were observed in SOD and CAT activities in the small intestine in the AA + Cr group ($p < 0.05$) compared to the AA group. SOD and CAT activities in large intestine of the AA + Cr group approached the C group levels.

AA administration caused a significant decrease ($p < 0.05$) in small and large intestine TAS levels compared to the Cr group, but a significant increase was observed in TOS levels ($p < 0.05$) compared to the Cr group. Although crocin therapy decreased TOS levels, it elevated TAS levels in small and large intestines in the AA + Cr group compared to the AA group. Table 1 and Table 2 present tissue oxidant-antioksidant parameters of all groups.

### Histology

The ileum sections of the C (Fig. 1A, B) and Cr (Fig. 1C, D) groups were evaluated for normal histological structure. We observed shortened villi and epithelial damage that reached the basal half of many villi and degenerated villi in the ileum sections of the AA group (Fig. 1E, F). We detected

**Table 1.** Ileum tissue oxidant-antioxidant parameters

| Groups | MDA (mol/g) | GSH (nmol/g) | SOD (U/g protein) | CAT (K/g protein) | TAS (mmol/l) | TOS (μmol/l) |
|---|---|---|---|---|---|---|
| C | 426.60 ± 36.66[a] | 948.04 ± 192.33[a] | 953.75 ± 169.55[a,b] | 26.15 ± 5.57[a,c] | 1.81 ± 0.34[a,b] | 3.98 ± 1.77[a] |
| Cr | 430.80 ± 48.99[a] | 1121.75 ± 175.47[a] | 752.23 ± 292.19[a] | 21.77 ± 6.38[a] | 2.08 ± 0. 11[a] | 3.57 ± 1.82[a] |
| AA | 528.40 ± 49.13[b] | 748.05 ± 67.10[b] | 1062.76 ± 76.51[b] | 37.4 5 ± 6.72[b] | 1.82 ± 0.11[b] | 11.94 ± 5.12[b] |
| AA + Cr | 461.11 ± 27.07[a] | 976.45 ± 132.62[a] | 862.58 ± 156.08[a] | 29.37 ± 5.37[c] | 2.01 ± 0.1[a.b] | 7.70 ± 2.83[a,b] |

Data are means ± SD, n = 10. MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; TAS, total antioxidant status; TOS, total oxidant status. Groups: C, control; Cr, crocin; AA, acrylamide; AA + Cr, acrylamide + crocin. Groups with different superscripts are significantly different.

**Table 2.** Colon tissue oxidant-antioxidant parameters

| Groups | MDA (nmol/g) | GSH (nmol/g) | SOD (U/g protein) | CAT (K/g protein) | TAS (mmol/l) | TOS (μmol/l) |
|---|---|---|---|---|---|---|
| C | 362.47 ± 51.60[a] | 629.46 ± 71.81[a,b] | 328.47 ± 83.80[a,b] | 28.19 ± 5.56[a] | 1.33 ± 0.03[a,b] | 5.44 ± 1.54[a,b] |
| Cr | 321.93 ± 88.92[a] | 732.02 ± 109.61[a] | 306.69 ± 106.98[a] | 26.75 ± 7.64[a] | 1.47 ± 0.08[a] | 4.63 ± 0.86[a] |
| AA | 549.74 ± 127.00[b] | 569.85 ± 141.36[b] | 421.89 ± 28.38[b] | 40.87 ± 6.65[b] | 1.27 ± 0.12[b] | 12.89 ± 4.87[b] |
| AA + Cr | 444.43 ± 121.85[a,b] | 695.13 ± 109.12[a,b] | 334.65 ± 72.07[a,b] | 34.87 ± 7.68[a,b] | 1.45 ± 0.08[a] | 7.13 ± 2.23[a,b] |

Data are means ± SD, n = 10. MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; TAS, total antioxidant status; TOS, total oxidant status. Groups: C, control; Cr, crocin; AA, acrylamide; AA + Cr, acrylamide + crocin. Groups with different superscripts are significantly different.

moderate inflammatory cell infiltration in the lamina propria and severe inflammatory cell infiltration in foci within the lamina propria. Deterioration of Lieberkühn crypt structures and localized crypt degeneration were observed. The total damage score was 14 for the AA group. Our findings of mucosal damage and degeneration indicated that absorption and secretion functions were impaired in the ileum.

In the AA + Cr group ileum, shortened and damaged villi, epithelial injury, Lieberkühn crypt injury and inflammation were observed and the total damage score (6) was reduced significantly compared to the AA group (Fig. 1G, H).

Normal histological structure was observed in colon sections of the C (Fig. 2A, B) and Cr groups (Fig. 2D). The AA group colon sections exhibited extensive degeneration of surface epithelium and Lieberkühn crypts, and total mucosa degeneration was observed in some sections compared to controls. Fibrosis and diffuse inflammatory cell infiltration were observed in degenerated mucosal areas. A significant reduction in goblet cell density in the surface epithelium was observed (Fig. 2E, F). The total damage score was 12 for the AA group.

We detected Goblet cell depletion, epithelial injury, mucosal fibrosis and Lieberkühn crypt injury in the colon sections of AA + Cr group (Fig. 2G, H). Inflammatory cell infiltration and total damage score (5), however, were decreased significantly compared to the AA group. Tables 3 and 4 present tissue damage scores.

## Discussion

AA is highly toxic; it is soluble in water and can be dispersed readily throughout the body. AA is formed when protein- and carbohydrate-rich foods are cooked at temperatures above 120° C. Endreich and Freidman (Erdreich and Friedman 2004) reported that AA formation depends on the time and temperature of the cooking process. AA is generated in baked, fried and grilled foods, but not in boiled foods (Mottram et al. 2002).

The most important function of the small intestine is to absorb nutrients and transfer them to the circulation. The intestinal mucosa can suffer oxidative stress injury by exposure to ROS produced by metal ions (iron, copper etc.), bacterial metabolites, xenobiotics, oxidants and oxidized food residues in the lumen (Halliwell et al. 2000). Oxidative stress is the result of alteration of the oxidant-antioxidant equilibrium in favor of oxidants. The antioxidant defense system normally controls free radical formation (Halliwell and Jm 2015).

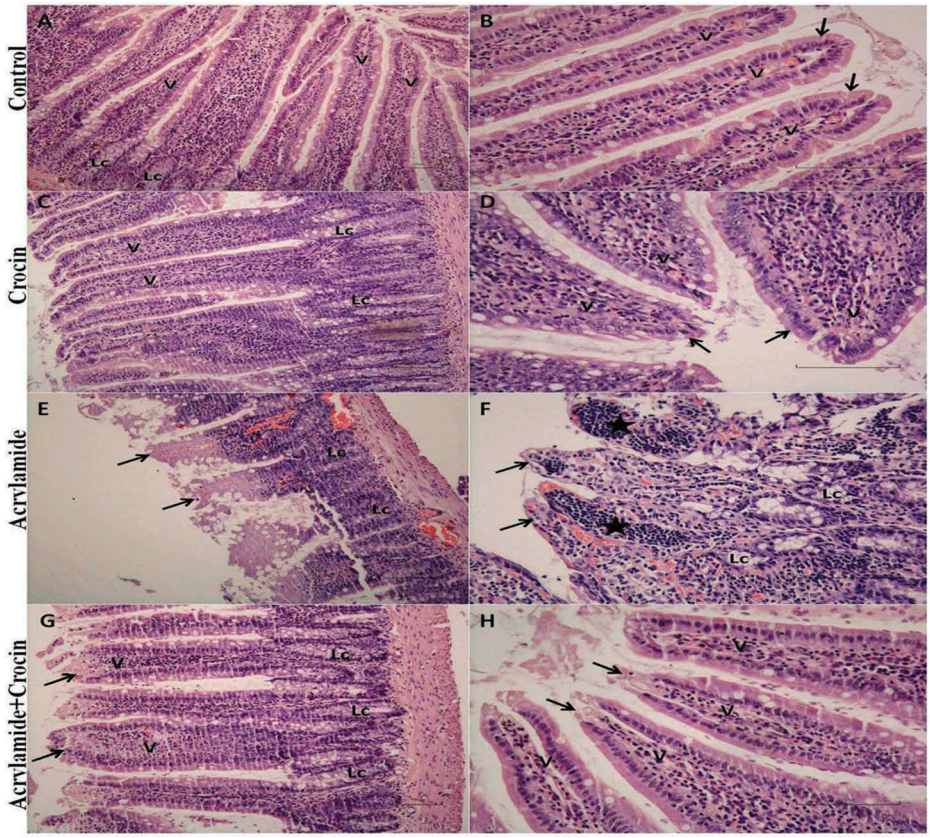


**Fig. 1.** Photomicrographs of small intestine. A) C group. V, villus; Lc, Lieberkühn crypt. H & E. 10 ×. B) C group. V, villus; arrow, villus surface epithelium. H & E. 20 ×. C) Cr group. V, villus; Lc, Lieberkühn crypt. H & E. 10 ×. D) Cr group. V, villus; arrow, villus surface epithelium. H & E. 20 ×. E) AA group. Arrow, villus degeneration; Lc, degenerated Lieberkühn crypt. H & E. 10 ×. F) AA group. Arrow, villus degeneration and loss of surface epithelium; asterisk, inflammatory cell infiltration in villus mucosa; Lc, degenerated Lieberkühn crypt structure. H & E. 20 ×. G); AA + Cr group. V, degeneration in villus; Lc, Lieberkühn crypt structure. H & E. 10 ×. H) AA + Cr group. Arrow, degeneration in villus. H & E. 20×.

We found statistically significant increases in SOD and CAT activities, and MDA and TOS levels in the small and large intestine that were exposed only to AA, while GSH and TAS levels decreased owing to AA induced oxidative stress. We also found that SOD and CAT activities and the increased MDA and TOS levels due to AA exposure were decreased by crocin treatment that prevented tissue damage due to increased free radical formation and lipid oxidation. Our findings were consistent with earlier reports that MDA levels, and SOD and CAT activities increased and GSH decreased in liver tissue following exposure to carbon tetrachloride ($CCl_4$) (Gangarapu et al. 2013, Ozturk et al. 2003, Ranjbar et al. 2014). Mottram et al. (2002) reported that AA administration decreased liver GSH levels due to the oxidization of AA to N-acetyl-S-(2-carbomoylethyl) cysteine by the GST enzyme. We found that GSH levels in intestinal tissues were reduced significantly by AA administration; our findings were consistent with the earlier report (Mottram et al. 2002). Like another earlier report (Zheng et al. 2007), we demonstrated that crocin protected intestinal tissues by decreasing amounts of ROS.

Hosseinzadeh et al. (2005) reported that saffron extract and crocin reversed the oxidative damage caused by ischemia-reperfusion induction in rat kidneys. These investigators demonstrated that saffron extract and crocin reduced free radical induced lipid oxidation and MDA levels due to their antioxidant properties.

Consistent with our findings, Sun et al. (2014) reported that crocin improved CDDP-induced oxidative stress by reducing MDA levels and restoring normal levels of GSH and antioxidant enzymes including SOD, CAT and GSH-Px. Ghadrdoost et al. (2011) demonstrated that saffron and crocin

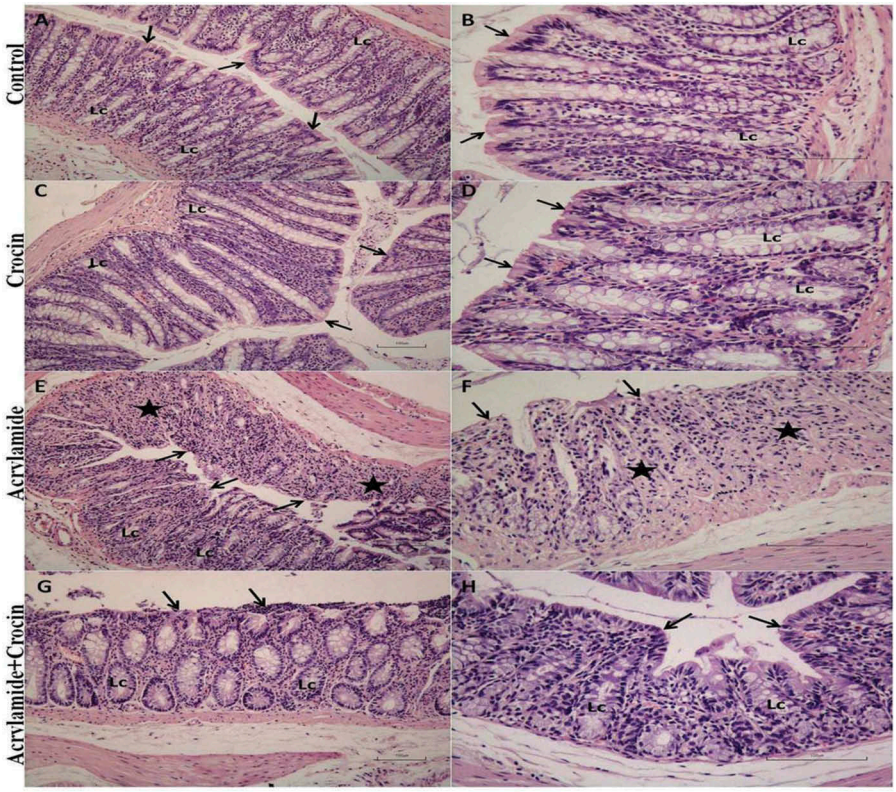


**Fig. 2.** Photomicrographs of large intestine tissue. A) C group. Lc, Lieberkühn crypt; arrow, colon surface epithelium. H & E. 10×. B) C group. Lc, Lieberkühn crypt; arrow, colon surface epithelium. H & E. 20×. C) Cr group. Lc, Lieberkühn crypt; arrow, colon surface epithelium. H & E. 10×. D) Cr group. Lc, Lieberkühn crypt; arrow, colon surface epithelium. H & E. 20×. E) AA group. Asterisk, colon mucosa degeneration; arrow, surface epithelium damage; Lc, degenerated Lieberkühn crypt structure. H & E. 10×. F) AA group. Asterisk, colon mucosa degeneration; arrow, surface epithelium damage; Lc, degenerated Lieberkühn crypt structure. H & E. 20×. G) AA + Cr group. Arrow, colon surface epithelium damage and intraepithelial inflammatory cellular infiltration; Lc, Lieberkühn crypt. H & E. 10 x, H) AA + Cr group. Arrow, colon surface epithelium; Lc, Lieberkühn crypt. H & E. 20 x.

**Table 3.** Ileum tissue damage scores

| Groups | Mean | Minimum | Maximum |
|---|---|---|---|
| $C^a$ | 0 | 0 | 0 |
| $Cr^a$ | 0 | 0 | 0 |
| $AA^b$ | 10.5 | 8 | 14 |
| $AA+Cr^c$ | 3.5 | 2 | 6 |

Groups: C, control; Cr, crocin; AA, acrylamide; AA + Cr, acrylamide + crocin. Groups with different superscripts are significantly different.

**Table 4.** Colon tissue damage scores

| Groups | mean | minimum | maximum |
|---|---|---|---|
| $C^a$ | 0 | 0 | 1 |
| $Cr^a$ | 0 | 0 | 1 |
| $AA^b$ | 10 | 9 | 12 |
| $AA+Cr^c$ | 3 | 2 | 5 |

Groups: C, control; Cr, crocin; AA, acrylamide; AA + Cr, acrylamide + crocin. Groups with different superscripts are significantly different.

regulated oxidative markers in the hippocampus; their findings were consistent with ours. It has been reported that crocin caused increased liver GSH levels and reduced MDA levels in rats treated with cisplatin (Sun et al. 2014), cyclophosphamide (Jnaneshwari et al. 2013) or diazinon (Lari et al. 2015). Therefore, owing to its antioxidant properties, crocin reduced cellular damage to levels close to the control groups. Lieshout et al. (1998) reported that when antioxidant-rich nutrients were consumed, the conjugation of toxic substances with GSH and their excretion were facilitated.

We demonstrated that crocin protected ileum and colon tissues against AA induced lipid oxidation and partially reversed the tissue damage. Crocin significantly reduced MDA levels that

had been elevated due to AA exposure. The effect of crocin on tissues appears to be due to its antioxidant properties (Assimopoulou et al. 2005).

Paolini et al. (2001) reported that beta carotene supplementation stimulated the activity of CYP isoforms, CYP1A1, CYP3A1, CYP2E1 and CYP2B1. Crocin is a carotenoid that may exert an inhibitory effect on CYP2E1, which causes the conversion of AA to glycidamide, which is more toxic than AA. Crocin also might protect intestinal tissues by inhibiting CYP2E1 isoenzyme activities (Tsujimoto et al. 2009). Therefore, inhibition of the CYP2E1 enzyme may have prevented oxidative damage by inhibiting the conversion of AA into the toxic metabolite, glycidamide, in intestinal tissues.

We demonstrated that crocin exerts protective effects on AA induced small and large intestine damages by inhibiting oxidative stress. Crocin exhibits antioxidant effects due to its free oxygen radical scavenging properties. Elevated MDA, TOS, SOD and CAT levels due to AA exposure decreased as a result of crocin treatment, while GSH and TAS levels increased. We demonstrated the therapeutic effect of crocin on AA induced damage to intestinal tissues and we believe that this effect might be due to its inhibition of the activity of the CYP isoenzymes.

## Funding

This work was supported by Research Fund of the Karabuk University [KBU-BAP-15/2-YL-010];

***Declaration of interest:*** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

## ORCID

ME Erdemli http://orcid.org/0000-0003-4596-7525
M Gul http://orcid.org/0000-0002-5721-8778
B Yigitcan http://orcid.org/0000-0002-7910-4595
H Gozukara Bag http://orcid.org/0000-0003-1208-4072
Z Aksungur http://orcid.org/0000-0002-9002-6604
E Altinoz http://orcid.org/0000-0002-3991-9773

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