Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d2fo02782h

Faba beans with enhanced antioxidant activity ameliorate acetic acid-induced colitis in experimental rats

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Faba beans are among the legumes that are of the greatest importance due to their high nutritional value. In addition to the essential nutrients that faba beans contain, they also contain bioactive compounds such as phenolics and flavonoids that are considered as potent natural antioxidants. Ulcerative colitis (UC) is an inflammatory bowel disease in which oxidative stress plays an essential role in the pathophysiology. The aim of the current study was to evaluate the antioxidant activity of faba bean seeds harvested from plants grown from seeds pre-treated with selenium, garlic husk extract and/or lemon peel extract and to evaluate their *in vivo* effects in a rat model of UC. 54 female rats were divided randomly into nine groups (n = 9). All groups were given the different tested treatments 14 days prior to UC induction using acetic acid (intra-rectal injection of 2 ml, 4% v/v in saline). Our results revealed that the treatment of faba bean seeds with a mixture of selenium, garlic husk extract and lemon peel extract before planting led to a significant increase in selenium, nitrogen, potassium, total protein, phenolic and flavonoid content in the harvested faba bean seeds with a subsequent enhancement of their antioxidant capacity. Consumption of such faba beans showed potential protective and therapeutic effects during experimental colitis by reducing colonic oxidative stress and increasing colonic antioxidant defense mechanisms. Further research is required to understand the mechanisms by which faba beans influence colitis, their effects on various inflammatory biomarkers and their impact on the severity of colitis in humans.

Received 19th September 2022,
Accepted 24th October 2022

DOI: 10.1039/d2fo02782h

rsc.li/food-function

1 Introduction

Faba bean (*Vicia faba* L.) is one of the most important legumes with high nutritional value for humans and animals, due to its richness in many nutrients such as proteins, starch, dietary fibers, fatty acids, vitamins and minerals.[1,2] In addition, faba bean is considered a rich source of micronutrients called phytonutrients, which are secondary metabolites accumulated in different parts of plants.[3] These phytonutrients are bioactive compounds that act as natural antioxidants due to their high content of many functional phenolic and flavonoid compounds such as tannins, proanthocyanidins, L-3,4-dihydrophenylalanine (L-dopa), flavonols and flavones.[4,5] In humans, natural antioxidants can protect biological molecules such as lipids, proteins, and DNA against reactive oxygen and nitrogen species (ROS and RNS).[6]

Selenium (Se) is an essential micronutrient involved in many biological processes. An adequate daily intake of Se is important for the functional balance of many organs such as the thyroid, brain, muscles, prostate and testes.[7] Se acts as an antioxidant protecting cells and tissues from oxidative stress, and therefore sustaining redox status in cells. The antioxidant activity of Se is due to its incorporation into the structure of selenoproteins such as thioredoxin reductases (TrxR), glutathione peroxidases, selenoprotein P (SelP), selenoprotein F (SelF), selenoprotein S (SelS) and selenoprotein M (SelM).[8–10]

Se organic compounds are much more bioavailable than Se inorganic compounds. Accordingly, it should be pointed out that obtaining the daily requirement of selenium from foods with a high content of Se is much better than obtaining Se from supplements.[7,11]

Garlic (*Allium sativum* L.) is a common spice with many health benefits that are attributed to its various bioactive compounds, such as organic sulfides, saponins, phenolic compounds, and polysaccharides.[12,13] Garlic and its active ingredients, mainly phenols and saponins, have a certain antioxidant activity.[14] Moreover, the extract of garlic skins (peels or husks)

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has shown strong antioxidant activity due to its constituents such as phenylpropanoids.[15]

Lemon (*Citrus limon* L.) is an important medicinal plant that belongs to the Rutaceae family. Lemon peels exhibit a wide range of biological activities due to the abundance of bioactive constituents. Lemon peels contain phenolic compounds (phenolic acids and flavanones) that are responsible for various bioactivities such as antimicrobial, antioxidant and anticancer.[16]

Ulcerative colitis (UC) is one of the inflammatory bowel diseases characterized by inflammation and ulceration of the lining of the colon and rectum. The exact cause of UC remains unknown. Previously, diet and stress were thought to be the cause, but now it is known that these factors may exaggerate but do not cause UC. One possible cause is an uncontrolled immune system response against intestinal microbial flora. This abnormal immune response causes the immune system to attack the cells in the digestive tract as well.[17,18]

Oxidative stress plays a critical role in the pathogenesis of UC and its malignant progression to colorectal cancer (CRC).[19] Oxidative stress occurs if the generation of ROS exceeds the defensive capability of the antioxidant system in cells. This excessive ROS production causes lipid peroxidation, intestinal mucosal barrier damage, bacterial translocation, and an inflammatory response.[20] Accordingly, increasing the buffering capability of the antioxidant defense in the host using a diet with enhanced antioxidant activity may neutralize ROS overproduction and ameliorate UC.

In a previous study,[21] we successfully grew bean plants from grains pre-treated with selenium, garlic husk extract and lemon peel extract using a recent three-in-one approach. This new approach takes advantage of the nutritional value of the residue extracts in increasing the yield of the beans and enhancing their antioxidant activity as well as recycling these residues.

In the present study, we aim to evaluate the antioxidant activity of faba bean seeds harvested from plants grown from seeds pre-treated with selenium, garlic husk extract and/or lemon peel extract and to investigate the extent of their prophylactic effects in preventing acetic acid (AA)-induced colitis in experimental rats.

2 Materials and methods

2.1 Animals

The study was performed in accordance with the Guidelines for the Care and Use of Laboratory Animals,[22,23] the basis of the animal ethics guidelines of the Institutional Animals Ethics Committee (protocol no. 2011/186) and the basis of the Canadian Council on Animal Care guidelines. In the current study, a total of 54 female albino rats aged 6–8 weeks old and weighing approximately 90–120 g were obtained from the animal house, Agriculture Research Center, Cairo, Egypt. The rats were housed in plastic cages, and were provided a standard pellet diet and water *ad libitum*. Animals were housed at normal temperatures under a normal dark/light cycle [temperature (25 ± 2 °C) and 12 h light/dark cycle] and acclimatized for a period of 7 days to laboratory conditions.

2.2 Experimental design

This experiment included the following nine treatments using 6 experimental rats per each treatment: (1) the negative control group (NC) – in which rats were fed the basal diet; (2) the colitis positive control group (PC) – in which rats were fed the basal diet; (3) the bean group (B) – in which rats were fed the basal diet supplemented with untreated beans; (4) the selenium group (Se) – in which rats were fed the basal diet supplemented with beans pre-treated with selenium before planting; (5) the garlic group (G) – in which rats were fed the basal diet supplemented with beans pre-treated with garlic husk extract before planting; (6) the lemon group (L) – in which rats were fed the basal diet supplemented with beans pre-treated with lemon peel extract before planting; (7) the garlic + selenium group (G + Se) – in which rats were fed the basal diet supplemented with beans pre-treated with garlic husk extract and selenium before planting; (8) the lemon + selenium group (L + Se) – in which rats were fed the basal diet supplemented with beans pre-treated with lemon peel extract and selenium before planting; and (9) the garlic + lemon + selenium group (G + L + Se) – in which rats were fed the basal diet supplemented with beans pre-treated with lemon peel, garlic husk extracts and selenium before planting.

2.3 Induction of colitis

All groups were given the mentioned treatments for 14 days. Under light sedation with ether, colitis was induced on day 14 by intra-rectal injection of 2 ml of acetic acid (AA) (4% v/v in saline) using an elastic catheter (with an outer diameter of 2 mm).[24,25] Negative control animals underwent the same procedure using an equal volume of normal saline instead of AA solution.

2.4 Sample preparation

The rats were subjected to the previously mentioned treatments for a period of 60 days. At the end of the experiment, blood was withdrawn from the retro-orbital plexus and the serum was separated and stored at −20 °C for biochemical analysis. Animals were sacrificed, and the colons were removed, washed with normal saline and observed for macroscopic colitis assessment, and then cut into small portions and kept at −80 °C for biochemical analysis and histopathological investigations.

2.5 Evaluation of the disease activity index (DAI)

The clinical severity of colitis was evaluated through the calculation of DAI according to a previously described method.[26] The DAI uses a scoring system for evaluating the percentage of weight loss, stool consistency, and rectal bleeding. The following parameters were recorded daily: body weight loss (0, none; 1, 1–5%; 2, 6–10%; 3, 11–20%; and 4, >20%), diarrhea (0, normal; 1, soft stool but still formed; 2, very soft stool; 3, mild

diarrhea; and 4, severe diarrhea), and rectal bleeding (0, normal; 1, positive hemoccult; 2, visible blood traces in stool; 3, mild bleeding; and 4, severe bleeding). The DAI values were calculated according to this equation:

$$\mathrm{DAI} = \frac{\text{body weight loss score} + \text{diarrhea score} + \text{rectal bleeding score}}{3}$$

2.6 Macroscopic scoring

For about half of the rats, the entire excised colon was weighed and used for macroscopic scoring. The assessment of macroscopic injury was carried out by analyzing the presence or absence of colonic thickening, hyperemia, ulcers, necrosis, and adherence to nearby organs, according to previously described criteria.[27]

2.7 Histopathological investigations

Histopathological assessments were performed according to the method described by Bancroft and Gamble (2008).[28] Colon biopsies were fixed with 10% neutral buffer formalin and embedded in paraffin. The specimens were washed, dehydrated using alcohol, cleared in xylene and embedded in paraffin wax blocks. For histopathological assessments, 3 μm thickness sections were cut and stained with hematoxylin and eosin (H&E) and were mounted and observed microscopically for histopathological changes by an experienced pathologist in a blinded fashion.

2.8 Assessment of oxidative stress and the antioxidant indices in colon tissues

2.8.1 Colonic malondialdehyde (MDA). The colonic malondialdehyde (MDA) content was determined based on the method described by Ohkawa *et al.* (1979).[29] 2 ml of colon homogenate (20% w/v) supernatant was mixed with an equal amount of thiobarbituric acid TCA (10% w/v), frozen for 15 min, and then centrifuged at 20 000 rpm. Afterwards, 2 ml of TCA was mixed again with 2 ml of the supernatant. The solution was heated for 10 min at 100 °C and rapidly cooled at 0 °C for 5 min. The concentration was measured by spectrophotometry at 535 nm.

2.8.2 Colonic superoxide dismutase (SOD) activity. SOD activity in the colonic tissue homogenate was measured according the method described by Nishikimi *et al.* (1972).[30] SOD assay relies on the ability of SOD to inhibit the phenazine methosulphate-mediated reduction of the nitro-blue tetrazolium dye.

2.8.3 Colonic catalase (CAT) activity. CAT activity in the colonic tissue homogenate was assessed based on the method described by Aebi (1984).[31] CAT assay is a colorimetric method that depends on the measurement of the hydrogen peroxide (H_2O_2) substrate using a redox dye. The change in the color intensity at 570 nm is directly proportional to the CAT activity.

2.8.4 Colonic glutathione reductase (GR) activity. GR activity in the colonic tissue homogenate was assayed colorimetrically as described by Goldberg and Spooner (1983).[32] The method is based on the capacity of GR to catalyze the reduction of GSSG to GSH in the presence of NADPH, which is oxidized to $NADPH^+$. The decrease in absorbance at 340 nm was measured.

2.8.5 Colon reduced glutathione (GSH) level. The GSH level in the colon tissue homogenate was measured based on the method described by Beutler *et al.* (1963).[33] The method produces a yellow color by reducing 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) with glutathione (GSH). The reduced chromogen was directly proportional to the GSH concentration and its absorbance could be measured at 405 nm.

2.9 Determination of the total phenolic content (TPC) of the dry faba bean seed extract

The TPC was determined according to the Folin–Ciocâlteu colorimetric method referred to Gargouri *et al.* (2019).[34] Briefly, 1 mL of the ethanolic extract was mixed with 0.5 mL of the Folin–Ciocâlteu reagent in a test tube and thoroughly shaken. After 3 min, 1 mL of saturated Na_2CO_3 (20%) was added to the mixture and then the volume was made up to 10 mL with distilled water. The reaction was allowed to proceed for 1 h. A blank was prepared with 1 mL of distilled water instead of the sample. After 1 h, the absorbance was recorded at 725 nm using a spectrophotometer (UV-vis spectrophotometer UV 9100 B, LabTech). The concentration of the total soluble phenols was calculated using the standard curve of gallic acid. The total phenol concentration was expressed as μg equivalents of gallic acid per g DW of the sample.

2.10 Determination of the total nitrogen, total potassium, total selenium and total soluble protein content of the dry faba bean seed extract

Faba bean seeds were oven-dried at 65 °C, and then wet digested using a mixture of H_2SO_4 and H_2O_2 according to the method outlined by Cottenie *et al.* (1982).[35] The total nitrogen content in the dried faba bean seeds was determined by the micro-Kjeldahl method using 5% boric acid and 40% NaOH as described by Black (1965).[36] The total potassium content was determined using a flame photometer.[37] The total selenium content was determined using ICP mass spectrometry.[38] The total soluble protein concentration was quantified by the method of Bradford[39] using bovine serum albumin (BSA) as a standard.

2.11 Determination of the total flavonoid content (TFC) of the dry faba bean seed extract

The TFC was determined by the aluminum chloride colorimetric assay as described previously.[40] An aliquot of 1 mL of the ethanolic extract was added to 4 mL of distilled water in a 10 mL volumetric flask. Then, 0.3 mL of 5% $NaNO_2$ was added. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. At the 6th min, 2 mL of 1 M NaOH was added and the total volume was made up to 10 mL with distilled water. The solution was mixed well and the absorbance was measured against the blank at 510 nm. The concentration of total flavonoids was calculated using the standard curve of quercetin and was

expressed as μg of quercetin equivalent per g DW of the sample.

2.12 Determination of the antioxidant capacity of the dry faba bean seed extract

The antioxidant activity of the dry faba bean seed extract was determined based on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity as described by Alexandra *et al.* (2019).[41] In the DPPH assay, 1 mL of the sample was mixed with 0.5 mL of 50 μM DPPH in ethanol and kept in the dark for 30 min. The absorbance of the mixture was measured at 517 nm. A vitamin C standard (5–30 μg mL^{-1}) was used as the positive control. The radical scavenging activity was determined based on the percentage inhibition of absorbance, which was calculated using the following formula:

$$\%\ \text{inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100,$$

where A_{control} is the absorbance of the reaction without the sample and A_{sample} is the absorbance of the sample.

Lower absorbance indicates higher DPPH radical scavenging activity. The IC_{50} value is defined as the concentration of the sample required to scavenge 50% of DPPH. Therefore, lower IC_{50} indicates higher antioxidant activity.

2.13 Statistical analysis

The SPSS statistical package 21.0 (SPSS Inc., Chicago, Illinois, USA) was used for all statistical analyses. All data were presented as means ± standard deviation. One-way ANOVA was used to analyze differences between means for normally distributed values, followed by *post hoc* analysis. Correlations were evaluated using the Pearson correlation coefficient. All *p*-values were two-sided and the probability value, $p < 0.05$, was considered statistically significant.

3 Results

3.1 Effect of feeding with different types of beans on AA-induced alterations in disease severity and colonic macroscopic damage

The clinical severity of colitis was evaluated for each experimental group. The DAI uses a scoring system for evaluating the percentage of weight loss, stool consistency, and rectal bleeding. The DAI scores of the positive control (PC) rats were significantly higher than those of the negative control (NC) group ($p < 0.001$; Fig. 1A). However, compared with the PC group, rats fed with the different types of beans showed significant reductions in the DAI scores, especially those of the Se and G + L + Se groups ($p < 0.001$; Fig. 1A). Furthermore, macroscopic injury was evaluated with scores ranging from 0 to 10 points. The NC group scored below 1, while the PC group showed major damage such as increased colonic thickening, necrosis, ulcers, and adherence to adjacent organs. Feeding with different types of beans significantly decreased the intensity of all of the macroscopic damage especially in the Se, L + Se and G + L + Se groups ($p < 0.001$; Fig. 1A and C).

3.2 Effect of feeding with different types of beans on AA-induced histopathological changes in rat colon tissues

Fig. 2 shows the results of hematoxylin and eosin staining. The analysis of the Swiss-roll sections of the NC group revealed the normal histology of the colon wall; it consisted of tunica mucosa (mucosal crypts) that was formed of simple columnar epithelium and lamina propria that contained glands with basally situated nuclei and numerous goblet cells. Tunica mucosa rested on muscularis mucosa, and then it was submucosa and finally the muscular wall of the colon. The PC group showed severe damage with many ulcerated areas of the mucosa with minimal remnants of the crypts, which indicated hemorrhage and inflammatory cell infiltration. Also, the submucosa showed large areas of hemorrhage and inflammatory cell infiltration. The B group showed ulceration and destruction of the colon mucosa with necrosis in the glands and extensive inflammatory cell infiltration. The best protective action of a single agent was observed in the Se group. Few sections showed mild mononuclear inflammatory cell infiltration in the colonic mucosa, and some sections were apparently normal. The colon sections from the G group showed the least improvement. Some sections exhibited exaggerated mucus secretion with cystically dilated glands. The submucosa showed inflammatory edema. Some other sections showed extensive glandular necrosis with heavy inflammatory cell infiltration mainly by neutrophils. Both mucosa and submucosa were infiltrated by dense inflammatory cells. The L group showed mildly affected mucosa that appeared infiltrated by a few inflammatory cells, while the submucosa showed extensive inflammatory reaction. An extensive inflammatory reaction was clearly observed in the colonic wall of animals from this group. Some severely affected sections exhibited marked mucosal damage and ulceration. Some sections showed cystically dilated crypts. Regarding the groups treated using more than one protective agent, they exhibited a synergistic action in alleviating the induced colon damage. The G + Se group showed an apparently normal colon wall in most examined sections. Only mild inflammatory cell infiltration was observed around the glands. Concerning the colon sections from the L + Se group, all examined sections appeared apparently normal, except one case exhibiting mucosal damage and inflammatory cell infiltration of both mucosa and submucosa. Cystically dilated glands with exaggerated mucus production was also a quite common finding. The best protective action was achieved in the G + L + Se group. Apparently normal mucosa was observed in almost all examined sections. Mild mucosal and submucosal inflammatory reactions were also detected. A few sections showed cystically dilated glands with increased mucus secretion.

3.3 Effect of feeding with different types of beans on the colonic oxidative and antioxidant profiles in acetic acid (AA)-induced ulcerative colitis

We evaluated the effect of feeding with different types of beans on oxidative stress and antioxidant defense in colon tissues.

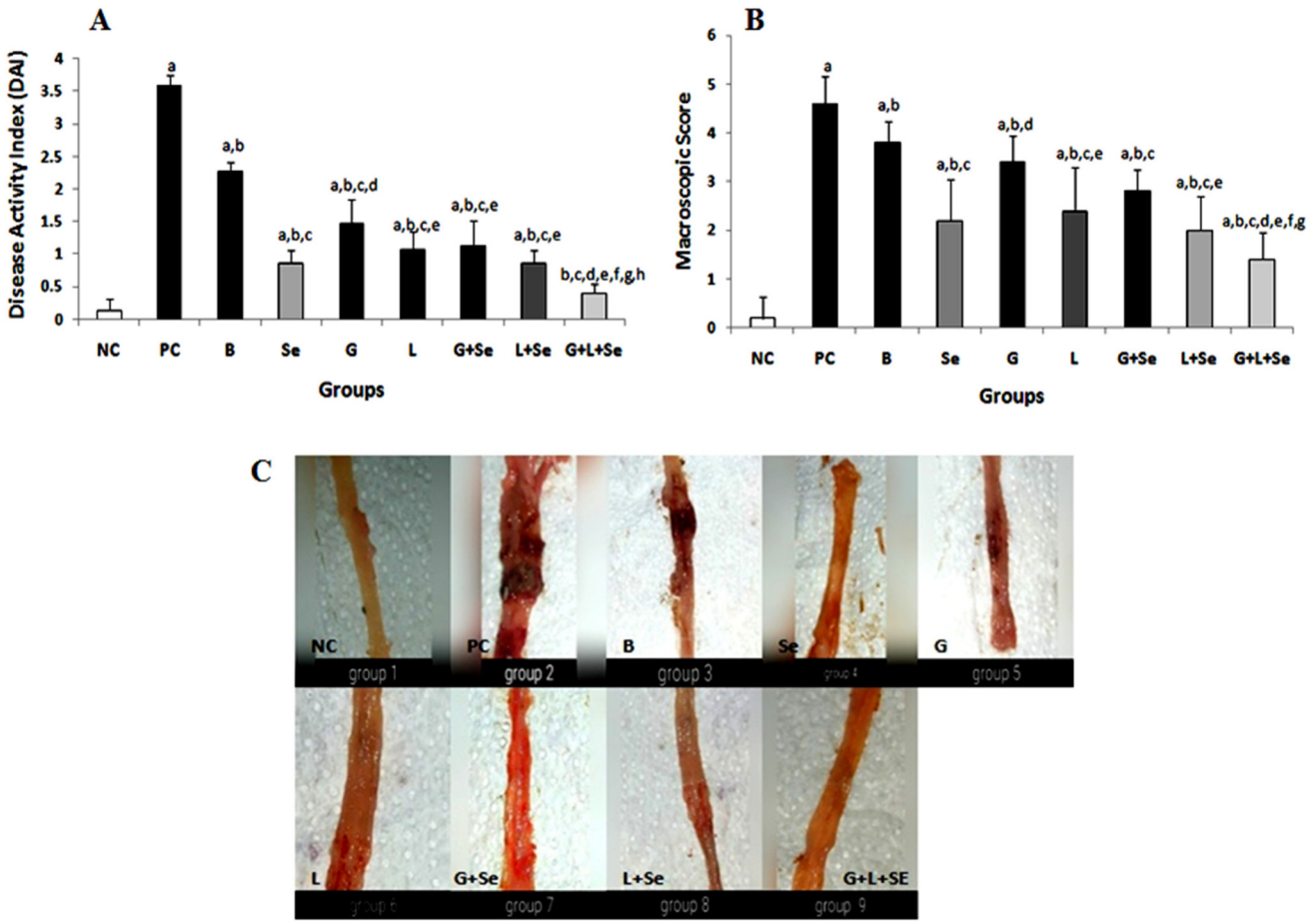

Fig. 1 Effect of feeding with different types of beans on acetic acid (AA)-induced alterations in disease severity and colonic macroscopic damage. (A) Disease activity index (DAI) scores of rat colons in different groups. (B) Quantification of the colon damage macroscopic scoring in different groups. (C) Photographic evaluation of the colon. Negative control (NC) group: colon without morphological alterations; positive control (PC) group: colon shows severe shortening and thickening with severe hyperemia and extensive tissue necrosis; bean (B) group: colon shows moderate shortening and thickening, with severe hyperemia and extensive tissue necrosis; selenium (Se) group: colon shows mild shortening and thickening with mild hyperemia; garlic (G) group: colon shows moderate shortening and thickening with moderate hyperaemia and tissue necrosis; lemon (L) group: colon shows moderate shortening and thickening with mild hyperaemia; garlic + selenium (G + Se) group: colon shows moderate shortening and thickening with moderate hyperaemia; lemon + selenium (L + Se) group: colon shows mild shortening and thickening with mild hyperemia; garlic + lemon + selenium (G + L + Se) group: colon shows only mild hyperaemia. Each value represents the mean ± SD (n = 6). Significant difference at $p < 0.001$. a: Significant *vs.* the NC group. b: Significant *vs.* the PC group. c: Significant *vs.* the B group. d: Significant *vs.* the Se group. e: Significant *vs.* the G group. f: Significant *vs.* the L group. g: Significant *vs.* the G + Se group. h: Significant *vs.* the L + Se group.

The results, presented in Table 1 and Fig. 3, show that in the positive control (PC) group, colonic MDA was increased by 2.7-fold compared to the negative control (NC) group ($p < 0.001$), indicative of increased oxidative stress in AA-induced colitis. On the other hand, rats fed different types of beans, especially those in the selenium (Se) and G + L + Se groups showed a significant decrease in the levels of colonic MDA by 31.4% and 29.6%, respectively, when compared to the PC group ($p < 0.001$).

In the PC group, the GRD and GSH activity levels in the colon tissues were lower than those in the NC group by 41.5% and 59.3%, respectively ($p < 0.001$), while the activities of SOD and CAT were increased by 1.37- and 1.6-fold, respectively ($p < 0.001$). Feeding with different types of beans had protective effects against these changes. Rats fed beans in the Se and G + L + Se groups showed increased SOD activity by 1.16- and 1.12-fold, respectively, with significant differences compared to the PC group. With regard to the colonic GSH level and GRD activity, rats fed beans in the G + L + Se, Se, L + Se, and G + Se groups showed significantly increased GSH levels by 3.23-, 2.18-, 1.8-, and 1.4-fold, respectively, compared to the PC group ($p < 0.001$). Moreover, colonic GRD activity significantly increased by 2.27-, 2.37-, 1.63- and 1.45-fold, respectively, compared to the PC group ($p < 0.001$). Regarding colonic CAT activity, rats fed beans in the Se, G and G + L + Se groups showed significantly increased CAT activity by 2-, 1.16- and 1.1-fold, respectively, compared to the PC group ($p < 0.001$).

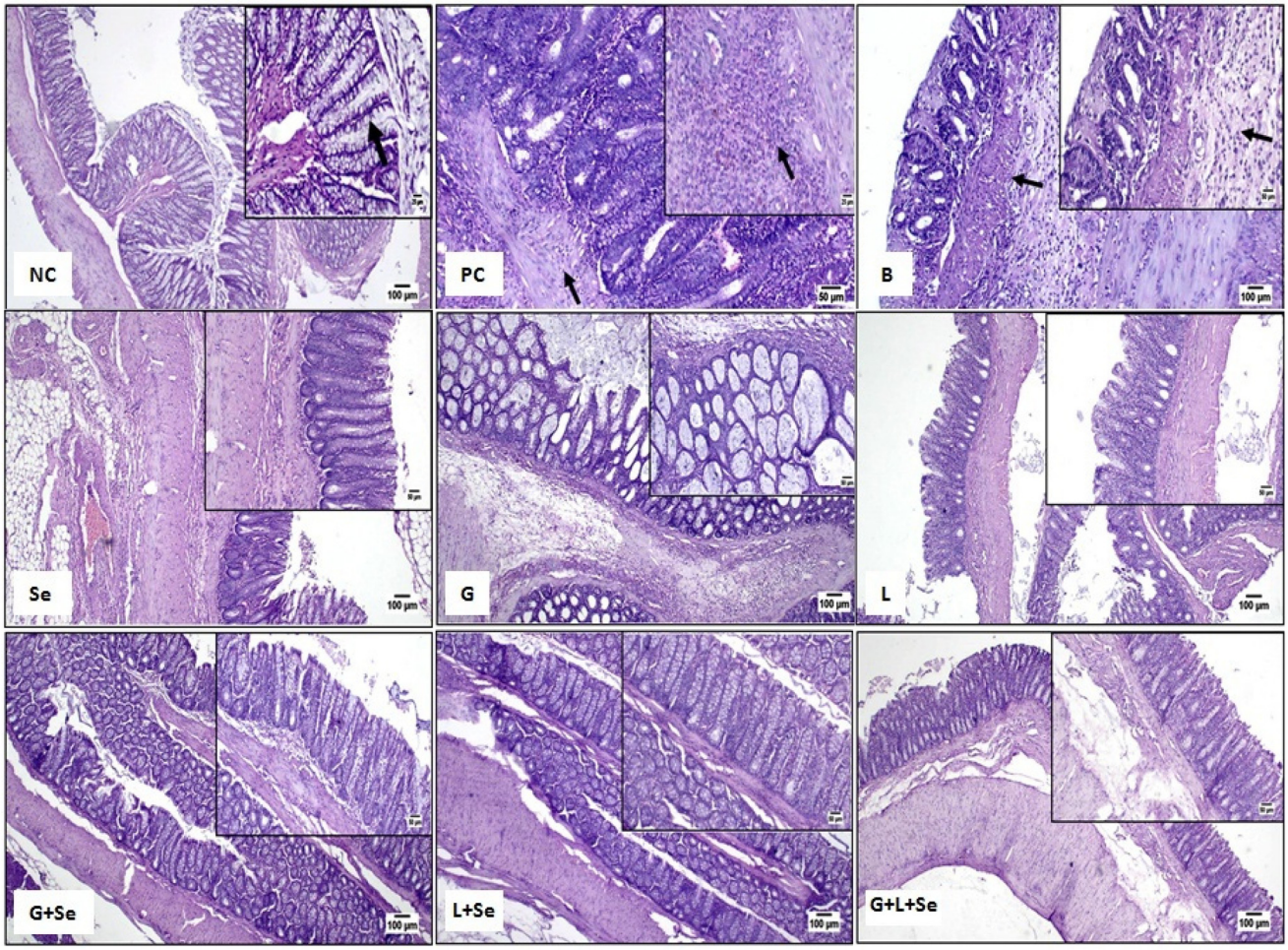

Fig. 2 Effect of feeding with different types of beans on acetic acid (AA)-induced histopathological changes in rat colonic tissues. Photomicrographs of the Swiss-roll sections of the colons of the negative control (NC) group: showing a normal structure of the colon wall. Inset shows a higher magnification of the crypts, showing many goblet cells and the absorptive simple columnar cells (arrow). The positive control (PC) group: showing distorted mucosa with remnants of crypts (black arrow) and mononuclear inflammatory cell infiltration of the submucosa (arrow). The inset shows a higher magnification of the submucosa with mononuclear inflammatory cells. The bean (B) group: showing complete destruction and necrosis of the colonic mucosa with submucosal edema and mononuclear inflammatory cell infiltration. Inset shows a higher magnification of the submucosa, showing submucosal edema and mononuclear inflammatory cell infiltration and some inflammatory cells including neutrophils. The selenium (Se) group: showing apparently normal colon mucosa with an extended inflammatory reaction to the serosa. The garlic (G) group: showing exaggerated mucus secretion with cystic dilatation of the gland with an extensive inflammatory reaction in the submucosa. Inset shows a higher magnification of exaggerated cystic dilatation of the gland and mononuclear inflammatory cell infiltration. The lemon (L) group: showing inflammatory cell infiltration in the mucosa with cystic dilatation. The garlic + selenium (G + Se) group: showing an apparently normal colon wall. The inset shows a higher magnification, showing mild inflammatory cell infiltration in the colonic mucosa. The lemon + selenium (L + Se) group: showing apparently normal colonic mucosa. The inset shows a higher magnification, showing mild inflammatory cell infiltration in the colonic mucosa. The garlic + lemon + selenium (G + L + Se) group: showing an apparently normal colon wall. The inset shows a higher magnification, showing mild inflammatory cell infiltration in the colonic mucosa.

3.4 Effect of different treatments on the total phenolic content (TPC), total flavonoid content (TFC), total nitrogen (N) content, total potassium (K) content, total selenium content (Se), total soluble protein content and the antioxidant capacity of the dry faba bean seed extract

The levels of total phenolic and total flavonoid content in the ethanolic extracts of dry faba bean seeds are shown in Fig. 4. Faba bean seeds from the G + L + Se and L + Se treatments showed the highest level of phenolic compounds compared to the control bean group (B) and the other treatments with high significant difference ($p < 0.001$) (Fig. 4A). On the other hand, G + L + Se, L + Se and Se treatments significantly increased the total flavonoid content of faba bean seeds compared to the control bean group (B) and the other treatments with high significant difference ($p < 0.001$) (Fig. 4B).

All treatments increased the total N and K contents of dry faba bean seeds compared to the control bean group (B) with high significant difference ($p < 0.001$) (Fig. 4C). Also, the Se content of dry faba bean seeds was increased in all treatments,

Table 1 Effect of feeding with different types of beans on the colonic oxidative and antioxidant profiles in acetic acid (AA)-induced ulcerative colitis

	NC	PC	B	Se	G	L	G + Se	L + Se	G + L + Se	Statistics
L-MDA (nmol g^{-1} tissue)	8.922 ±	$24.07^{(a)}$ ±	$18.91^{(a,b)}$ ±	$16.5^{(a,b,c)}$ ±	$19.2^{(a,b,d)}$ ±	$21.1^{(a,b,c,d,e)}$ ±	$19.64^{(a,b,d,f)}$ ±	$19.31^{(a,b,d,f)}$ ±	$16.94^{(a,b,c,e,f,g,h)}$ ±	*F*: 13.21, *P*:
	0.434	1.80	0.319	0.987	1.616	4.43	2.38	1.619	0.377	0.000
SOD (U mg^{-1} protein)	1.964 ±	$2.707^{(a)}$ ±	$1.961^{(b)}$ ±	$3.142^{(a,b,c)}$ ±	$2.40^{(a,b,c,d)}$ ±	$2.578^{(a,b,c,d,e)}$ ±	$2.094^{(a,b,c,d,e,f)}$ ±	$2.738^{(a,c,d,e,f,g)}$ ±	$3.025^{(a,b,c,d,e,f,g,h)}$ ±	*F*: 124.4, *P*:
	0.023	0.010	0.049	0.156	0.049	0.0179	0.0171	0.0167	0.1052	0.000
CAT (U mg^{-1} protein)	0.0023 ±	$0.0037^{(a)}$ ±	$0.0036^{(a)}$ ±	$0.0074^{(a,b,c)}$ ±	$0.0043^{(a,b,c,d)}$ ±	$0.004^{(a,b,c,d,e)}$ ±	$0.0034^{(a,b,d,e,f)}$ ±	$0.0036^{(a,d,e,f)}$ ± 3.8	$0.0041^{(a,b,c,d,g,h)}$ ±	*F*: 248.5, *P*:
	8×10^{-5}	4.5×10^{-5}	1.4×10^{-4}	3.7×10^{-4}	9.1×10^{-5}	5.7×10^{-5}	5×10^{-5}	$\times 10^{-5}$	1.3×10^{-4}	0.000
GSH (mmol mg^{-1} protein)	0.784 ±	$0.319^{(a)}$ ±	$0.376^{(a,b)}$ ±	$0.696^{(a,b,c)}$ ±	$0.406^{(a,b,d)}$ ±	$0.406^{(a,b,d)}$ ±	$0.449^{(a,b,c,d,e,f)}$ ±	$0.574^{(a,b,c,d,e,f,g)}$ ±	$1.033^{(a,b,c,d,e,f,g,h)}$ ±	*F*: 504.5, *P*:
	0.0081	0.001	0.0095	0.0354	0.011	0.0034	0.0056	0.0075	0.0365	0.000
GRD (U mg^{-1} protein)	2.767 ±	$1.619^{(a)}$ ±	$2.096^{(a,b)}$ ±	$3.843^{(a,b,c)}$ ±	$2.185^{(a,b,d)}$ ±	$1.895^{(a,b,c,d,e)}$ ±	$2.351^{(a,b,c,d,e,f)}$ ±	$2.641^{(b,c,d,e,f,g)}$ ±	$3.689^{(a,b,c,d,e,f,g,h)}$ ±	*F*: 317.7, *P*:
	0.0283	0.0029	0.0496	0.1779	0.0287	0.0079	0.0261	0.0082	0.1154	0.000

Data are represented as mean ± SD, $P < 0.001$, $n = 6$. a: Significant *versus* the negative control (NC) group. b: Significant *versus* the positive control (PC) group. c: Significant *versus* the bean (B) group. d: Significant *versus* the selenium (Se) group. e: Significant *versus* the garlic (G) group. f: Significant *versus* the lemon (L) group. g: Significant *versus* the garlic + selenium (G + Se) group. h: Significant *versus* the lemon + selenium (L + Se) group. SOD: super oxide dismutase, GSH: reduced glutathione, GRD: glutathione reductase, CAT: catalase, MDA: malondialdehyde.

especially in the G + L + Se treatment compared to the control bean group (B) with high significant difference ($p < 0.001$) (Fig. 4D). The G + L + Se, L + Se and Se treatments significantly increased the total protein content of dry faba bean seeds compared to the control bean group (B) and the other treatments with high significant difference ($p < 0.001$) (Fig. 4E).

To determine the antioxidant capacity, a DPPH radical scavenging capacity assay was employed to test the antioxidant capacity of the phenolic and flavonoid contents of the dry faba bean seed extract and the results are shown in Fig. 4F. The $IC_{50\%}$ value (defined as the concentration of the sample required to scavenge 50% of DPPH) was chosen to indicate the antioxidant activity. Therefore, a lower $IC_{50\%}$ indicates higher antioxidant activity. The data obtained indicated that the antioxidant capacity of seeds from the G + L + Se, L + Se and Se treatments was significantly higher than that of seeds from the control bean group (B) and the other treatments with a high significant difference ($p < 0.001$).

The correlations between the antioxidant capacity ($IC_{50\%}$ value), TPC, TFC, N content, K content, Se content and protein content were established, and the correlation coefficients (r) are tabulated in Table 2. A significant positive correlation was found between TPC and TFC ($r = 0.923$, $P < 0.01$), TPC and N content ($r = 0.555$, $P < 0.01$), TPC and protein content ($r = 0.888$, $P < 0.01$), TVC and K content ($r = 0.629$, $P < 0.01$), TVC and protein content ($r = 0.937$, $P < 0.01$), N content and K content ($r = 0.659$, $P < 0.01$), N content and Se content ($r = 0.779$, $P < 0.01$), N content and protein content ($r = 0.627$, $P < 0.01$), K content and Se content ($r = 0.755$, $P < 0.01$), K content and protein content ($r = 0.665$, $P < 0.01$) and Se content and protein content ($r = 0.579$, $P < 0.01$). Significant negative correlations were observed between the antioxidant capacity ($IC_{50\%}$ value) and TPC, TVC, nitrogen content, potassium content and protein content ($r = -0.836$, -0.896, -0.588, -0.651 and -0.827, respectively, $P < 0.01$).

3.5 Relationship between the TPC, TFC, N content, K content, total Se content, total soluble protein content and the antioxidant capacity ($IC_{50\%}$) of the dry faba bean seed extract and oxidative stress and the antioxidant indices in colon tissues

The results shown in Table 3 indicate that no significant correlations were found between TPC, TFC, N content, K content, total Se content, total soluble protein content and the antioxidant capacity ($IC_{50\%}$) of the dry faba bean seed extract and both colonic MDA level and CAT activity ($p > 0.05$). Conversely, significant positive correlations were found between the TPC, TFC, K content and protein content of the dry faba bean seed extract and colonic SOD activity, GSH level and GRD activity ($p < 0.001$). Furthermore, significant negative correlations were observed between the antioxidant capacity ($IC_{50\%}$ value) and colonic SOD activity, GSH level and GRD activity ($p < 0.001$).

4 Discussion

In a previous study,[21] we studied the effect of soaking bean seeds before planting in garlic peel extract and lemon peel

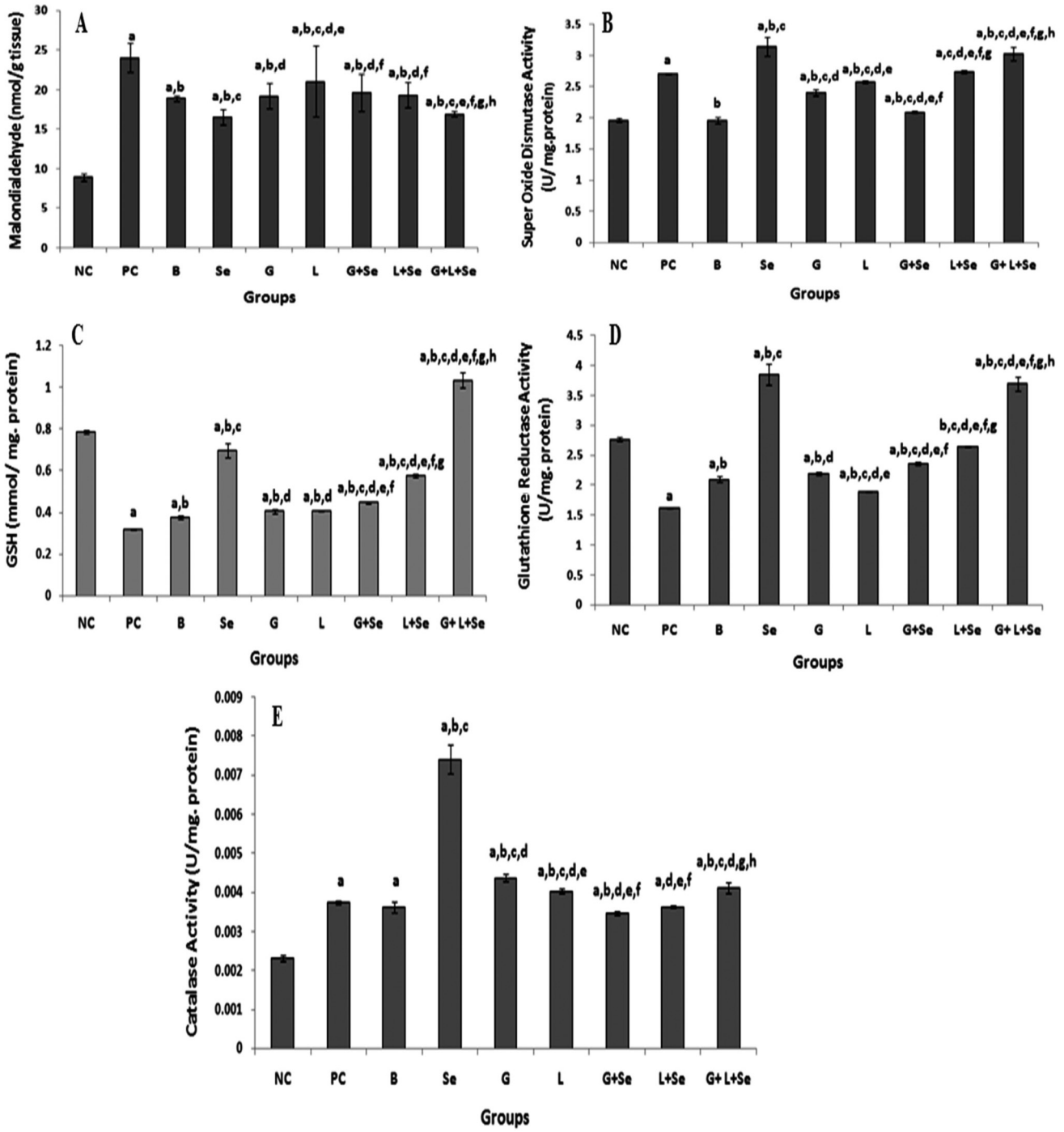

Fig. 3 Effect of feeding with different types of beans on the colonic oxidative and antioxidant profiles in acetic acid (AA)-induced ulcerative colitis. (A) Effect on the colonic malondialdehyde level, (B) effect on colonic superoxide dismutase activity, (C) effect on the colon reduced glutathione (GSH) level, (D) effect on colonic glutathione reductase activity and (E) effect on colonic catalase activity. Data are represented as mean ± SD, $P < 0.001$, $n = 6$. a: Significant *versus* the negative control (NC) group. b: Significant *versus* the positive control (PC) group. c: Significant *versus* the bean (B) group. d: Significant *versus* the selenium (Se) group. e: Significant *versus* the garlic (G) group. f: Significant *versus* the lemon (L) group. g: Significant *versus* the garlic + selenium (G + Se) group. h: Significant *versus* the lemon + selenium (L + Se) group.

extract with or without selenium on increasing fresh and dry weights, plant height, and nitrogen and potassium content, in addition to the effect on TFC and TVC during different growth stages, which was reflected in the final productivity.

In the current study, we have evaluated the antioxidant activity of faba bean seeds harvested from plants grown from seeds after being subjected to the previously mentioned soaking treatments and investigated the extent of their prophy-

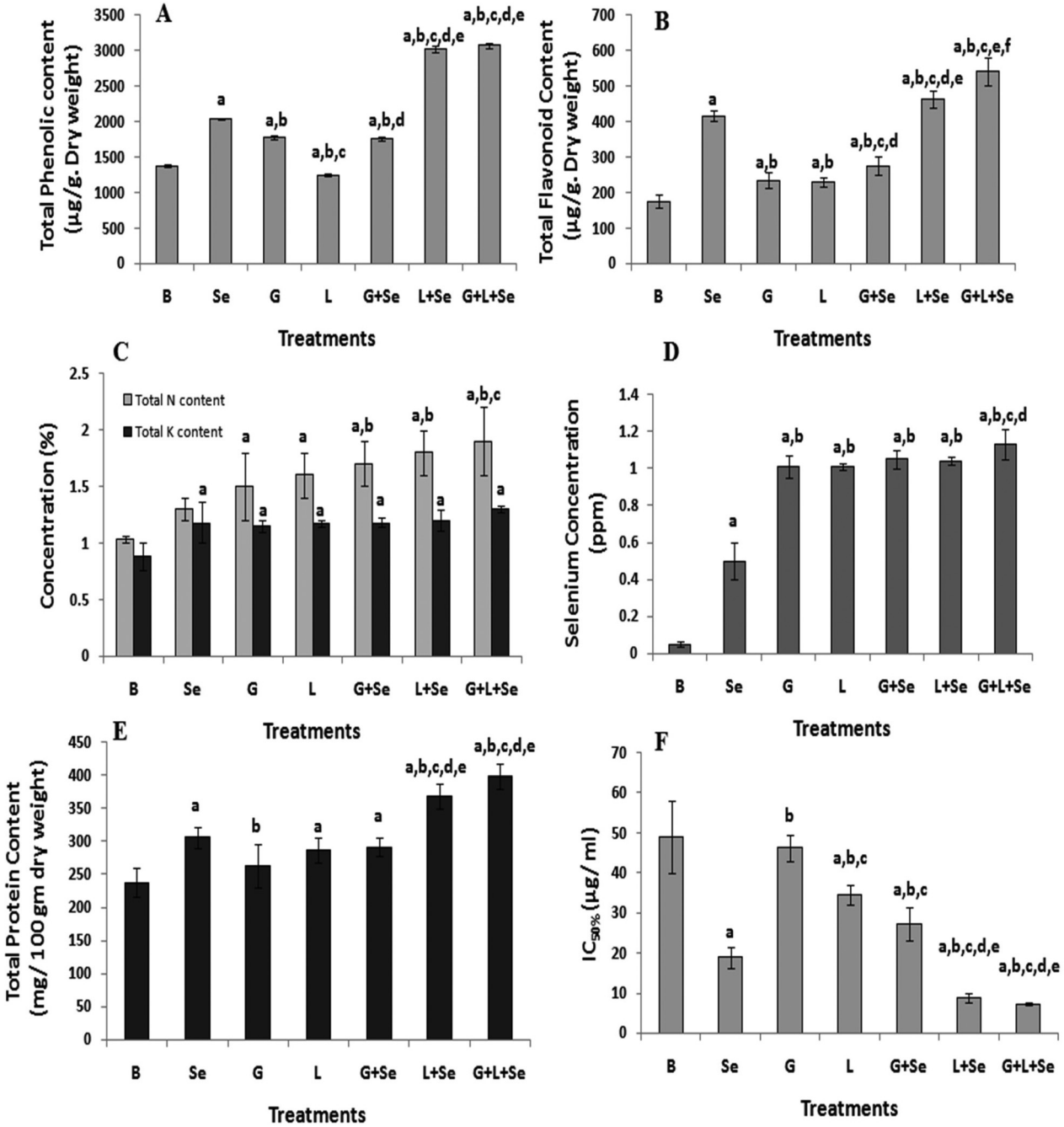

Fig. 4 Effect of different treatments on (A) the total phenolic content (TPC), (B) total flavonoid content (TFC), (C) total nitrogen and potassium contents, (D) total selenium content, (E) total protein content and (F) the antioxidant capacity ($IC_{50\%}$) of the dry faba bean seed extract. Data are represented as mean ± SD, $P < 0.001$. a: Significant *versus* the untreated beans (B). b: Significant *versus* the selenium pre-treated beans (Se). c: Significant *versus* the garlic husk extract pre-treated beans (G). d: Significant *versus* the lemon peel extract pre-treated beans (L). e: Significant *versus* the garlic husk extract + selenium pre-treated beans (G + Se). f: Significant *versus* the lemon peel extract + selenium pre-treated beans (L + Se).

lactic effects in preventing acetic acid (AA)-induced colitis in experimental rats. The AA-induced colitis model usually mimics the pathogenesis of human UC.[42,43]

Herein, clinical manifestations of colitis were evaluated for the experimental groups. The DAI was used to assess the percentage weight loss, stool consistency, and rectal bleeding. The obtained results showed that the rats fed with the different types of beans showed significant reductions in the DAI scores, especially in the Se and G + L + Se groups. Regarding macroscopic injury, feeding with different types of beans significantly reduced the severity of all macroscopic damage, especially in the Se, L + Se and G + L + Se groups.

An AA-induced colitis model can represent several histopathological features that are similar to human ulcerative

Table 2 Correlation analysis of the total phenolic content (TPC), total flavonoid content (TVC), nitrogen (N) content, potassium (K) content, selenium (Se) content, protein content and antioxidant capacity ($IC_{50\%}$)

	TPC	TVC	N content	K content	Se content	Protein content	$IC_{50\%}$
TPC	1.000 (—)	0.923** (0.000)	0.555** (0.009)	0.530* (0.013)	0.440* (0.046)	0.888** (0.000)	−0.836** (0.000)
TVC	0.923** (0.000)	1.000 (—)	0.492* (0.023)	0.629** (0.002)	0.415 (0.061)	0.937** (0.000)	−0.896** (0.000)
N content	0.555** (0.009)	0.492* (0.023)	1.000 (—)	0.659** (0.001)	0.779** (0.000)	0.627** (0.002)	−0.588** (0.005)
K content	0.530* (0.013)	0.629** (0.002)	0.659** (0.001)	1.000 (—)	0.755** (0.000)	0.665** (0.001)	−0.651** (0.001)
Se content	0.440* (0.046)	0.415 (0.061)	0.779** (0.000)	0.755** (0.000)	1.000 (—)	0.579** (0.005)	−0.499* (0.041)
Protein content	0.888** (0.000)	0.937** (0.000)	0.627** (0.002)	0.665** (0.001)	0.579** (0.005)	1.000 (—)	−0.827** (0.000)
$IC_{50\%}$	−0.836** (0.000)	0.896** (0.000)	−0.588** (0.005)	−0.651** (0.001)	−0.499* (0.041)	−0.827** (0.000)	1.000 (—)

**Correlation is significant at the 0.01 level (2-tailed).

Table 3 Correlation analysis between the TPC, TFC, N content, K content, Se content, protein content and the antioxidant capacity ($IC_{50\%}$) of the dry faba bean seed extract and the oxidative stress and antioxidant profiles in colon tissues

	MDA	SOD	CAT	GSH	GRD
TPC	−0.326 (0.149)	0.608** (0.003)	0.007 (0.976)	0.778** (0.000)	0.643** (0.002)
TVC	0.385 (0.085)	0.807** (0.000)	0.271 (0.234)	0.839** (0.000)	0.824** (0.000)
N content	−0.51 (0.825)	0.313 (0.167)	−0.271 (0.235)	0.417 (0.060)	0.168 (0.467)
K content	−0.164 (0.478)	0.611** (0.003)	0.161 (0.486)	0.551** (0.010)	0.454* (0.039)
Se content	0.159 (0.491)	0.305 (0.179)	−0.265 (0.247)	0.287 (0.207)	0.039 (0.867)
Protein content	−0.162 (0.484)	0.679** (0.001)	0.011 (0.964)	0.805** (0.000)	0.616** (0.003)
$IC_{50\%}$	0.333 (0.140)	−0.713** (0.000)	−0.175 (0.448)	−0.768** (0.000)	−0.698** (0.000)

**Correlation is significant at the 0.01 level (2-tailed). TPC: total phenolic content, TVC: total flavonoid content, N content: nitrogen content, K content: potassium content, Se content: selenium content, MDA: malondialdehyde, SOD: super oxide dismutase, CAT: catalase, GSH: reduced glutathione, GRD: glutathione reductase.

colitis, such as mucosal ulceration in which neutrophils infiltrate into the lamina propria and intestinal crypts.[42] Other histological features include the depletion of goblet cells.[43] Our findings showed that feeding with different types of beans significantly improved all the histopathological findings. In this regard, the G + L + Se group exhibited dramatic preventive and curative achievements, which was significantly different from the other groups. Based on these results, we conclude that consuming faba bean seeds pre-treated with a mixture of lemon peel, garlic husk extract and selenium before planting has prophylactic effects in rats with AA-induced colitis.

It has been previously reported that soaking seeds before planting in Se solution increases the rate of selenium uptake and its quantity in the final crop.[44] Moreover, Se supplementation increases the antioxidant activity of plants.[45,46] This is clearly evident in this study, where the different soaking treatments led to a significant increase in the Se content of dry faba bean seeds. Also, a significant positive correlation was found between the Se content and the antioxidant capacity of the dry faba bean seed extract.

In this study, no significant correlation was found between the Se content of dry faba bean seeds and the antioxidant indices in the colon tissues (SOD activity, GSH level and GRD activity). However, there was a significant correlation between the Se content and the total protein content, potassium content and antioxidant capacity ($IC_{50\%}$) of dry faba bean seeds, which are all significantly related to the antioxidant indices in the colon tissues. This may confirm the indirect relationship between the Se content of dry faba bean seeds and the antioxidant indices in the colon tissues.

As for garlic, previous studies showed that garlic is a source of antioxidants (phenols and flavonoids) and contains various growth-promoting compounds such as organo-sulphur compounds (allicin and diallyl disulphide) that improve the growth and yield of faba bean plants.[47,48] Phenolics and flavonoids are two of the major bioactive substances present in citrus fruits such as lemon, with a higher concentration in the peels than in the fruits.[49] Citrus phenolics and flavonoids are powerful antioxidants and potent free radical scavengers that help in the prevention of diseases that occur due to ROS.[50] In the current study, soaking faba bean seeds in garlic and/or lemon peel extract mixed with Se solution before planting significantly increased the TPC, TVC, total N content, total K content, total Se content and total protein content and finally the antioxidant capacity ($IC_{50\%}$) of dry faba bean seeds.

Faba beans are known to be rich in proteins and contain abundant dietary fibers that could contribute to anti-IBD activities through modulating gut microbiota and preventing gut dysbiosis.[51,52] A previous study conducted by Papoutsis *et al.*[53] investigated the role of protein and fiber fractions of faba beans for colonic health and microbiota composition in a low-grade inflammation mouse model. They found that faba bean fractions had minor effects on inflammatory parameters and colonic microbiota. Studying the effect of dietary intake of faba bean seeds on colonic microbiota was beyond the scope of the current study and the main concern was the effect on

the antioxidant indices in colon tissues. A significant correlation was found between the total protein content of dry faba bean seeds and the antioxidant indices in the colon tissues. This effect of faba bean proteins may be attributed to the amino acid profiles or the presence of bioactive peptides produced during the digestion of plant proteins as previously reported.[54,55]

ROS play an essential role in the pathophysiology of UC. In fact, UC patients exhibit lower antioxidant capacity and reveal greater levels of oxidative DNA damage than healthy individuals.[56,57] SOD, an endogenous antioxidant enzyme, converts superoxide to H_2O_2 in the colonic epithelium, while GSH, a non-enzymatic antioxidant, captures ROS and is converted to the oxidized form GSSG. GSH reductase (GRD) keeps GSH in its reduced state.[58] MDA is an end product of the lipid peroxidation process, which induces metabolic aberrations and results in cross-links with DNA proteins with consequent DNA breaks.[59] Such an oxidative imbalance stimulates the inflammatory cells to produce peroxynitrite, which establishes oxidative stress in UC.[60] The results of the current study are in agreement with these findings, as AA administration led to a remarkable oxidative imbalance. Feeding with pre-treated faba bean seeds, especially in the Se, L + Se and G + L + Se groups, decreased oxidative stress (decreased colonic MDA) and increased antioxidant defense mechanisms (increased colonic GSH, GRD, SOD and CAT) as a protective approach. Likewise, treatment with antioxidants such as *N*-acetylcysteine, the GSH precursor,[61] or the flavonoid quercetin[62] reduced colitis-induced oxidative stress.

Phenolics and flavonoids are among the most important phytonutrients contained in faba beans. These compounds occur ubiquitously in plant-based diets or medicinal plants, and faba beans are rich in these compounds.[63] Previous studies reported different therapeutic effects of phenolics and flavonoids due to their natural anti-oxidant, anti-carcinogenic,[64] anti-ulcer,[65] anti-inflammatory,[66] immunomodulatory,[67] and anti-microbial[68] activities.

In order to show the impact of the different treatments on the phenolic and flavonoid content of faba bean seeds, the TPC, TFC and antioxidant capacity of the dry faba bean seed extract were determined. The obtained results showed high levels of phenolic and flavonoid contents of faba bean seeds in different groups and this confirms the fact that faba bean is a rich source of phenolic and flavonoid compounds. These results are consistent with those reported previously for faba bean seeds.[69,70] Interestingly, the G + L + Se, L + Se and Se treatments, respectively, increased the TPC, TVC and antioxidant capacity in faba bean seeds as compared to the untreated beans (control group).

Correlations between the TPC and TFC and antioxidant capacity of the dry faba bean seed extract were analyzed. The obtained data revealed that the TPC and TFC in the faba bean seed extract strongly influenced the antioxidant capacity of the extract. Moreover, significant correlations were found between the TPC, TFC and antioxidant capacity of the dry faba bean seed extract and the antioxidant indices in the colon tissues (SOD activity, GSH level and GRD activity). Therefore, phenolic and flavonoid compounds may contribute to disease attenuation through their ability to reduce oxidative stress. In addition, previous studies, using various experimental colitis models, found down-regulation of pro-inflammatory cytokines in animals fed pure phenolic compounds.[71,72] Since faba beans are a rich source of phenolic compounds,[69,70] it is possible that the reduction in colitis symptoms observed in this study is due to the down-regulation of pro-inflammatory cytokines, but this needs to be investigated in further studies.

5 Conclusions

In conclusion, it became clear to us that planting faba bean seeds pre-treated with a mixture of selenium, garlic husk extract and lemon peel extract increased the faba beans' phenolic, flavonoid, Se, N, K and protein content with a subsequent enhancement in their antioxidant capacity. Consumption of such faba beans has potential protective and therapeutic effects during experimental colitis, as it reduces colonic oxidative stress and increases colonic antioxidant defense mechanisms. Further research is required to understand the mechanisms by which such faba beans influence colitis, their effects on various inflammatory biomarkers and their impact on the severity of colitis in humans.

Author contributions

Salwa M. El-sayed and Mona I. Nossier conceived and designed the research, performed the research and acquired the data; Ahmed I. Nossier analyzed and interpreted the data and drafted the manuscript. All authors were involved in revising the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors thank Prof. Dr Ahmed Abdel-Fattah, full-time professor of soil fertility and plant nutrition, Department of Soil and Water, Faculty of Agriculture, Ain Shams University, for reviewing this work. This work is self-funded.

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