Original Article

Investigating the Impact of Selected B Vitamins (B1, B2, B6, and B12) on Acute Colitis Induced Experimentally in Rats

Niloufar Dalayeli, Valiollah Hajhashemi[1], Ardeshir Talebi[2], Mohsen Minaiyan[1]

Department of Pharmacy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran, [1]Department of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Clinical Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: Malnutrition and lack of micronutrients are seen in many patients with ulcerative colitis (UC). Considering that vitamins play an important role in preventing or protecting against inflammation at the metabolic, physiological, and pathological levels, this research was aimed to investigate the effect of a number of vitamin B groups in improving UC. **Methods:** Experimental colitis was induced by rectal administration of acetic acid (3%) in male Wistar rats, and mega doses of thiamine (20 mg/kg), riboflavin (15 mg/kg), pyridoxine (30 mg/kg), and cyanocobalamin (250 μg/kg) alone or in combination were administered intraperitoneally for 5 days. In another group, normal dose (1/10 of the above-mentioned doses) of four vitamins in combination was administered for 15 days (started 10 days before colitis induction). Colon tissues were weighted and evaluated in terms of macroscopic, microscopic, and biochemical markers. **Results:** Normal dose of four vitamins in combination form and mega dose of thiamine caused a significant increase in the body weight of animals. All treatments except for pyridoxine (mega dose) diminished ulceration index, total colitis index, and colon weight compared to the control group. Myeloperoxidase (MPO) activity and malondialdehyde (MDA) levels decreased significantly in all treatment groups. **Conclusions:** The anti-inflammatory and anti-ulcerative properties of selected B vitamins improved experimental colitis regardless of the dosage and duration of treatment. Despite its beneficial effect on biochemical markers, pyridoxine had the least effect in reducing the pathological features of colitis. More studies are needed to confirm the effect of these vitamins in the clinical setting of this disease.

Keywords: *Acetic acid, anti-inflammatory agents, colitis, rats, ulcerative, vitamin B complex*

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) with an unknown etiology that affects the colon and rectum.[1] There are many responsible factors for increasing susceptibility to UC, such as genetics, disorder of the immune system, environmental factors, and nutritional habits, including foods with saturated fats and low fibers.[2] Malnutrition and lack of micronutrients are common problems in more than half of these patients.[3] Reduction in food intake, anorexia, food abstinence, malabsorption, and nutrient-drug interactions are some of the various reasons that can lower vitamins and mineral absorption. The most common micronutrient deficiencies in patients with UC include iron, cyanocobalamin (vitamin B12), vitamin K, folic acid, selenium, pyridoxine (vitamin B6), and thiamine (vitamin B1), and thus, these patients are usually advised to be consumed multivitamins and specific mineral supplements.[3,4]

For treating mild-to-moderate UC, mesalazine and sulfasalazine are first-choice drugs. Corticosteroids that are given orally, rectally, or by injection are effective in moderate to severe cases. For suppressing the immune inflammatory response, drugs, such as azathioprine, 6-mercaptopurine, cyclosporine A, and anti-tumor necrosis factor alpha (TNF-α) drugs, are used. However, they can cause a wide range of side effects, such as allergic reactions, osteoporosis, bone marrow suppression, and serious hepatic damages.[1,2]

Considering the complications caused by drugs used in patients with UC and also the available evidences that show the protective effects of some food components against the inflammation associated with colitis, finding of alternative or complementary treatments, such as consuming dietary

How to cite this article: Dalayeli N, Hajhashemi V, Talebi A, Minaiyan M. Investigating the impact of selected B vitamins (B1, B2, B6, and B12) on acute colitis induced experimentally in rats. Int J Prev Med 2024;15:61.

Address for correspondence:
Prof. Mohsen Minaiyan, Department of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: minaiyan@pharm.mui.ac.ir

Access this article online

Website: www.ijpvmjournal.net/www.ijpm.ir

DOI: 10.4103/ijpvm.ijpvm_232_23

Quick Response Code:

supplements, vitamins, nutrients, and probiotics, is assumed to be effective and safe in the management of colitis.[3,5]

Vitamin B1 is a water-soluble vitamin and is absorbed in the ileum and jejunum by active and passive uptake.[5] Symptoms of thiamin deficiency mainly include fatigue, loss of appetite, constipation, digestive disorders, cramps, and muscular atrophy.[6] The analgesic and anti-inflammatory potential of thiamin has been investigated in one study and has shown remarkable effects in high doses.[7]

The role of riboflavin (vitamin B2) in the proper functioning of the immune system and its deficiency in inflammatory diseases has been investigated in several studies.[8,9] It has been shown that the oral consumption of riboflavin in rats is capable of creating an anti-inflammatory and anti-hyperalgesia effect against painful inflammatory stimuli.[10]

Vitamin B6 exists in three forms, among which pyridoxal 5'-phosphate (PLP) is the most biologically active compound. Decreased serum PLP levels are consistently observed with the inflammatory conditions in various tissues. In addition, serum PLP level has been shown to be a risk factor for chronic diseases and even some cancers, such as colon cancer.[11-13]

The causes of vitamin B12 deficiency in patients with UC include anemia, decreased absorption of cyanocobalamin due to the inflammation of the colon, decreased consumption of food sources of vitamin B12, liver dysfunction, and ileum resections.[3,5] In addition, in a study, the impact of cyanocobalamin has been shown in control of chronic and acute pain as well as its anti-inflammatory effect.[14]

Considering micronutrient deficiencies in UC patients and also the role of vitamins in reducing inflammation and modulating immune system responses, this study was conducted to investigate the ameliorative effect of selected B vitamins (B1, B2, B6, and B) at normal (daily recommended) and high (mega) doses in an animal experimental model of colitis.

Methods

Animals

Male Wistar rats (180-220 g) bred in the animal house of the Isfahan School of Pharmacy were used in this study. Animals were kept in the same situation and controlled condition of temperature (21-23°C), humidity (40% to 50%), 12/12-h light/dark cycles, and suitable diet. Every three rats were housed in one cage with free access to standard rodent chow pellets and tap water. All animal procedures were conducted according to the local ethics guidelines for research on animals and approved by the Research Committee of Isfahan University of Medical Sciences (IR.MUI.RESEARCH.REC.1400.281).

Drugs and chemicals

Dexamethasone and mesalazine powders were purchased from Iran Hormone Pharmaceutical Company (Tehran, Iran). Thiamin hydrochloride powder was purchased from Mofid Pharmaceutical Company. Riboflavin (as phosphate sodium) powder was supplied by Raha Pharmaceutical Company. Pyridoxine was purchased from Darou Pakhsh Pharmaceutical Company, and cyanocobalamin was supplied by Exir Pharmaceutical Company. The kit for malondialdehyde (MDA) assessment was purchased from Navand Salamat Corporation (Uremia, Iran). Orthodianisidine hydrochloride (ODZ) and hexadecyl trimethyl ammonium bromide (HTAB) were obtained from Sigma-Aldrich Company (Darmstadt, Germany).

Experimental groups

The rats were randomly divided into 10 groups, each group comprising six rats as follows:

Group 1 (Normal): Normal saline (2 mL) was injected rectally instead of acetic acid and the rats treated with vehicle (distilled water, 5 mL/kg/d, i.p.) for 5 days.

Group 2 (Control): Acetic acid (2 mL, 3%) was injected rectally and the rats treated with vehicle (5 mL/kg/d, i.p.), 2 h before colitis induction and continued for 5 days.

Groups 3: Colitis was induced and the rats treated with normal (recommended daily) dose of four vitamins (thiamin 2 mg/kg/d, riboflavin 1.5 mg/kg/d, pyridoxine 3 mg/kg/d, and cyanocobalamin 25 μg/kg/d) concurrently 10 days before colitis induction and after that continued for 5 days.[15]

Groups 4: Colitis was induced and the rats treated with mega (×10 of normal) dose of four vitamins (thiamin 20 mg/kg/d, riboflavin 15 mg/kg/d, pyridoxine 30 mg/kg/d, and cyanocobalamin 250 μg/kg/d) concurrently for 5 days started 2 h before colitis induction.[16]

Groups 5: Colitis was induced and the rats treated with mega dose of vitamin B1 (20 mg/kg/d, i.p.) for 5 days started 2 h before UC induction.

Groups 6: Colitis was induced and the rats treated with mega dose of vitamin B2 (15 mg/kg/d, i.p.) for 5 days started 2 h before UC induction.

Groups 7: Colitis was induced and the rats treated with mega dose of vitamin B6 (30 mg/kg/d, i.p.) for 5 days started 2 h before UC induction.

Groups 8: Colitis was induced and the rats treated with mega dose of vitamin B12 (250 μg/kg/d, i.p.) for 5 days started 2 h before UC induction.

Groups 9 and 10 (referencereference): Colitis was induced and the rats treated with dexamethasone (1 mg/kg, i.p.) or mesalazine (150 mg/kg, p.o.) for 5 days started 2 h before UC induction.

The animals were euthanized 24 h after the last treatment through inhalation of CO_2 in a special chamber.

Induction of colitis

Rats were fasted 24 h before induction of colitis with free access to water. They were anesthetized with desflurane inhalation, and then, 2 mL of 3% acetic acid was slowly infused into the distal colon using a suitable catheter. Before taking the catheter out, the rats were maintained in a head-down position for 40 sec. to prevent leakage of solution.[17]

Assessment of colon macroscopic damage

The rat's abdomen was opened, and the colon, 8 cm in length and 3 cm proximal to the anus, was excised. It was cut longitudinally, rinsed with normal saline, weighed, and fixed on a flat and white working sheet. Photographs of colon segments were taken by camera, and the ulcerated area was analyzed by the Fiji-32 Image Processor Program. The ulcer score was evaluated through the following scores: 0 = no ulcer; 1 = inflammation, edema, thickness, and superficial ulcer; 2 = bleeding and deep ulcer; and 3 = necrosis and/or perforation. For better comparison between the effects of interventions, the ulcer index, summing the ulcer score and the ulcer area, was measured too.[18]

Histopathologic assessment of colon

The incised fixed colon tissues were dehydrated and cleared in xylene. They were next embedded in paraffin routinely prepared to be 5–6 microns thick and were stained with hematoxylin and eosin (H and E) for light microscopy.[18] The histopathological assessment of ulceration in the colon samples was scored based on the inflammatory severity and extent, crypt damage, and leukocyte infiltration, and finally, total colitis index was calculated based on these parameters as described in previous works.[19]

Determination of myeloperoxidase (MPO) activity in colon tissue

First, 100 mg of the tissue was weighed and completely crushed. Then, it was homogenized in 10 mM potassium phosphate buffer (pH 7) containing 0.5% HTAB. The resulting mixture was sonicated for 10 sec. in an ice bath and centrifuged at 10,000 rpm for 30 min at 4°C; finally, H2O2 (0.1 mM) and ODZ (1.6 mM) were added to 0.1 ml of the supernatant solution of each centrifuged tube sample, and the absorbance of the sample was measured at 450 nm. MPO activity was calculated and reported as U/100 mg of wet colon tissue.[20]

Malondialdehyde (MDA) assessment of colonic tissue

According to the manufacturer's instruction (assay kit), potassium chloride solution of 1.15% was prepared and 1 mL was added to homogenized colon tissues (0.1 g). The homogenized samples were centrifuged for 10 min at 7500 rpm, and the obtained supernatant was used to measure MDA level, while the absorbance was determined at 532 nm.[21]

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) with Tukey as *post hoc* test. For analysis of weight changes, Student's t-paired test was applied. Mann-Whitney U-test was also used for storing data using Statistical Package for Social Science (SPSS) software (version 16). Findings were mainly reported as mean ± SEM (standard error of mean) except for the scoring data, which was displayed as the median (range). $P < 0.05$ was considered statistically significant.

Results

Body weight changes in rats

As shown in Table 1, weight loss caused by UC occurred in control rats ($P < 0.05$). The trend of weight loss also occurred in the groups treated with pyridoxine and dexamethasone, which was not significant ($P > 0.05$). Instead, the weight of rats in other groups increased, which was significant in the groups treated with combination of four vitamins at normal dose, thiamine at mega dose, and mesalazine (at least $P < 0.05$).

Macroscopic assessment

As shown in Table 2 and Figure 1, macroscopic observation in the control group showed maximum ulcer area, ulcer score, ulcer index, and colon weight, which are indicative of the highest level of injury produced by acetic acid compared to the normal group that showed no changes. Dexamethasone (1 mg/kg, i.p.)

Table 1: Body weight changes in rats with colitis treated with selected Vitamin B

Groups	Primary	Secondary	% change	*P*
Normal	181.7±11.7	206±15.9	12.1	<0.05
Control	195.2±7.8	173.7±26.5	-11.0	<0.05
4BND	192.0±11.3	217.3±13.6	11.6	<0.01
4BMD	184.5±4.5	194.0±8.3	5.4	NS
B1MD	186.7±7.5	202.2±13.7	7.6	<0.05
B2MD	194.2±7.4	208.0±20.0	6.6	NS
B6MD	186.8±9.3	176.3±23.5	-5.6	NS
B12MD	193.3±5.0	201.5±6.0	4.0	NS
Dex. 1	185.8±16.7	176.0±4.9	-5.2	NS
Mes. 150	183.3±9.0	211.7±5.2	13.4	<0.01

4BND: four B vitamins at normal dose (B1, B2, B6, and B12); 4BMD: four B vitamins at mega dose; B1MD: B1 vitamin at mega dose; B2MD: B2 vitamin at mega dose; B6MD: B6 vitamin at mega dose; B12MD: B12 vitamin at mega dose. Dex. 1: dexamethasone (1 mg/kg/d); Mes. 150: mesalazine (150mg/kg/d). Data are shown as mean±SEM. Student's paired *t*-test was used for analysis (n=6). NS: non-significant

and mesalazine (150 mg/kg, p.o.) as positive controls led to significant healing ($P < 0.001$) in all macroscopic parameters [Table 2]. All of the interventions were effective to attenuate assessed macroscopic parameters (at least $P < 0.05$) except the mega dose of pyridoxine (30 mg/kg) ($P > 0.05$).

Histological assessment

According to Table 3 and Figure 2, no histological damage was observed in the normal group. On the contrary, microscopic assessments in the control group in which colitis was induced by acetic acid and treated with normal saline revealed the highest degree of total colitis index as an estimate of inflammation extent and severity, crypt damage, and leukocyte infiltration. Dexamethasone and mesalazine as positive controls led to the reduction of total colitis index ($P < 0.001$) in colon lesions [Table 3]. All of the treatment groups, except the group which received mega dose of pyridoxine, reduced total colitis index (at least $P < 0.05$).

Biochemistry assessment (MDA and MPO)

There was a significant increase in colonic MPO activity and MDA level in the control group as compared to the normal group [Table 3]. All the interventions, including the mega dose of pyridoxine, reduced the levels of MDA and MPO activity in a significant manner (at least $P < 0.05$). Dexamethasone and mesalazine as reference drugs led to diminished MPO activity and MDA levels significantly ($P < 0.001$) [Table 3].

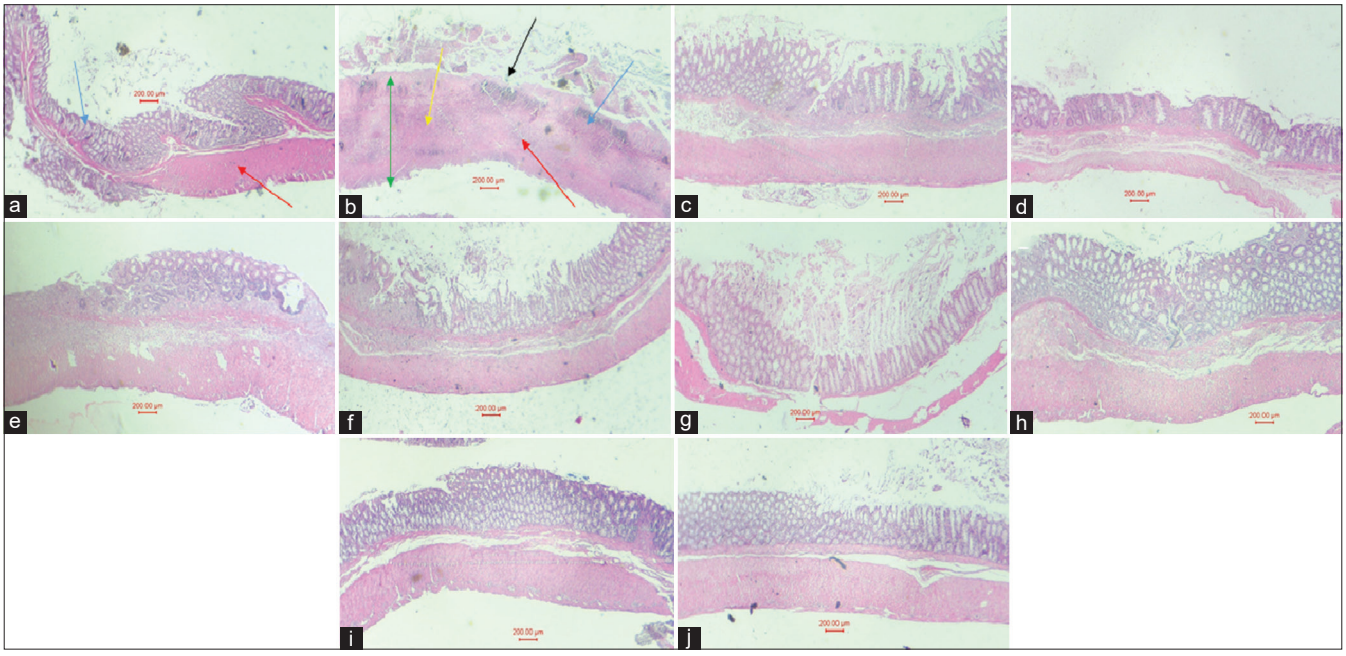

Figure 1: Macroscopic illustration of acetic acid-induced colitis in rats. (a) Normal; (b) control: colitis features, including thickness, edema, erythema, and necrosis of tissue, are evident; (c) normal dose of four vitamins (B1, B2, B6, and B12); (d) mega (×10 of normal) dose of four vitamins; (e) mega dose of vitamin B1; (f) mega dose of vitamin B2; (g) mega dose of vitamin B6; (h) mega dose of vitamin B12; (i) mesalazine (150 mg/kg); and (j) dexamethasone (1 mg/kg)

Table 2: Macroscopic parameters of colons of rats with colitis treated with selected vitamin B

Groups (doses)	Ulcer area (0–4)	Ulcer score (0–3)	Ulcer index (0–7)	Colon weight (mg)
Normal	0.0±0.0$^{###}$	0.0±0.0$^{###}$	0.0±0.0$^{###}$	408.3±65.2$^{###}$
Control	2.97±0.34	2.66±0.36	5.57±0.27	1230.1±89.4
4BND	1.04±0.18***	1.33±0.16***	2.35±0.23***	661.7±111.2***
4BMD	0.75±0.16***	1.25±0.13***	2.05±0.31***	703.3±64.1***
B1MD	1.65±0.21*	1.66±0.33*	3.49±0.22*	868.3±128.6*
B2MD	0.76±0.15***	1.25±0.13***	1.67±0.18***	791.6±68.5**
B6MD	1.54±0.29*	2.16±0.36	4.10±0.36	970.0±71.1
B12MD	0.87±0.11***	0.66±0.08***	1.55±0.13***	788.8±33.43**
Dex. 1	0.72±0.26***	0.66±0.11***	1.43±0.30***	743.3±34.7***
Mes. 150	0.83±0.13***	1.00±0.13***	1.83±0.27***	776.7±40.9**

Data are shown as mean±SEM. For score of ulcers, median (range) is represented. 4BND: four B vitamins at normal dose (B1, B2, B6, and B12); 4BMD: Four B vitamins at mega dose; B1MD: B1 vitamin at mega dose; B2MD: B2 vitamin at mega dose; B6MD: B6 vitamin at mega dose; B12MD: B12 vitamin at mega dose. Dex. 1: dexamethasone (1 mg/kg/d); Mes. 150: mesalazine (150 mg/kg/d). $^{###}P<0.01$: significant difference versus normal group, *$P<0.05$, **$P<0.01$, ***$P<0.001$: significant difference versus control group ($n=6$)

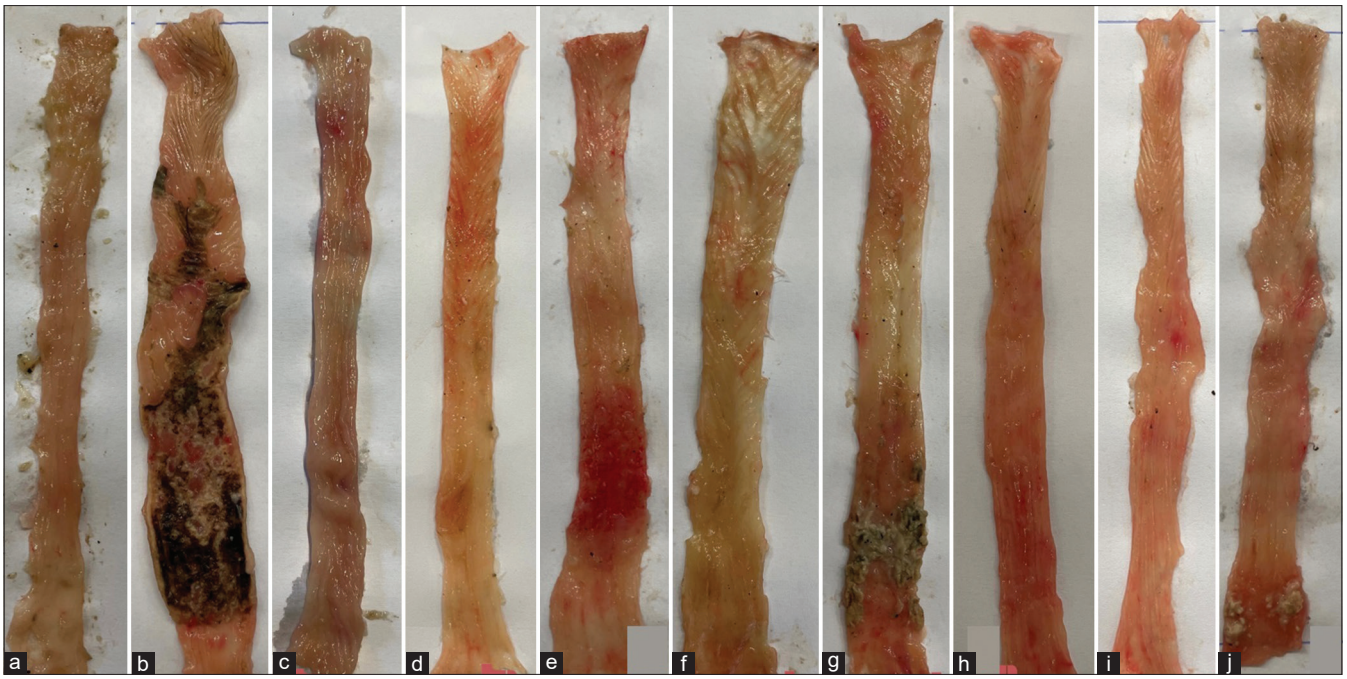

Figure 2: Microscopic presentation of acetic acid-induced colitis in rats. (a) Normal: mucosal and sub-mucosal layers as well as crypts are intact; (b) control: colitis features, including ulcer (black arrow), inflammation (red and green arrows), crypt damage (blue arrow), and leukocyte aggregation (yellow arrow) are evident; (c) normal dose of four vitamins (B1, B2, B6, and B12); (d) mega (×10 of normal) dose of four vitamins; (e) mega dose of vitamin B1; (f) mega dose of vitamin B2; (g) mega dose of vitamin B6; (h) mega dose of vitamin B12; (i) mesalazine (150 mg/kg); and (j) dexamethasone (1 mg/kg)

Table 3: Pathologic and biochemical parameters of colons of rats with colitis treated with selected vitamin B

Groups	Total colitis index (0–12)	MPO activity (U/100 mg)	MDA (nM/100 mg)
Normal	0.0±0.0	0.43±0.06	28.8±8.5
Control	12.0 (11-12)$^{###}$	1.95±0.16$^{###}$	176.2±16.5$^{###}$
4BND	6.0 (4-12)***	0.83±0.11***	89.5±7.2***
4BMD	6.2 (2-11)***	0.65±0.12***	51.2±7.1***
B1MD	7.66 (5-11)*	1.5±0.22*	112.2±13.6*
B2MD	6.5 (4-9)***	0.74±0.13***	82.9±7.6***
B6MD	10.0 (8-12)*	1.45±0.28*	101.3±7.9*
B12MD	8.5 (9-12)*	0.77±0.15***	61.3±9.7***
Dexamethasone	2.5 (1-7)***	0.49±0.12***	45.6±8.3***
Mesalazine	4.5 (4-8) ***	0.77±0.15***	55.5±8.4***

Data are shown as median (range) for total colitis index and mean±SEM for other parameters. 4BND: four B vitamins at normal dose (B1, B2, B6, and B12); 4BMD: four B vitamins at mega dose; B1MD: B1 vitamin at mega dose; B2MD: B2 vitamin at mega dose; B6MD: B6 vitamin at mega dose; B12MD: B12 vitamin at mega dose. Dexamethasone (1 mg/kg/d), mesalazine (150 mg/kg/d). $^{###}P<0.001$: significant difference versus normal group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$: significant difference versus control group (n=6)

Discussion

UC is a chronic IBD that affects the mucosa of the digestive tract. Unfortunately, in recent years, the visits of patients to the doctor due to IBDs have increased.[1] As inflammation and malnutrition are seen in colitis disease, the absorption and oral bioavailability of micronutrients and vitamins are greatly reduced in these patients.[3,5,22] However, there are many reports about anti-inflammatory and antioxidant properties of all types of vitamins from the B family.[6,14,23,24] Therefore, we decided to investigate the possible healing effect of four vitamins in this group consisting of B1, B2, B6, and B12. Acetic acid induction as an animal model of acute UC which has many similarities to human colitis was applied, and all the treatments were conducted by injection to rule out the lack of response due to vitamin malabsorption.[25]

Body weight loss seen in the control group was indicative that the disease was developed successfully. Among the reference groups, mesalazine caused a significant increase in body weight of rats compared to the control group, which showed its effectiveness in improving colitis and the general health of the animal. On the contrary, dexamethasone did not increase the animal's weight despite the clear improvement in tissue colitis indices. This effect of dexamethasone might be attributed to its catabolic properties as a corticosteroid.[26] Among the treatment groups, those who received mega dose of vitamin B1 or were treated with four vitamins (B1, B2, B6, and B12) at normal dose showed significant weight gain compared to the control group suggesting that the appetite and clinical manifestations of the disease were better improved in these two groups. Supporting this finding, there are reports that show that among B vitamins, vitamin B1 plays an important role in appetite control and its deficiency is associated with loss of appetite and body weight.[6]

Based on macroscopic and microscopic parameters, all treatment groups with selected B vitamins, except the mega dose of vitamin B6, were effective to reduce the evaluated colitis parameters in a significant manner. As the vitamin B6 at mega dose had no meaningful effect on the main indices of colitis, it is likely that this vitamin had no significant role in the efficacy of combination of four vitamins (normal dose or mega dose) examined in this study. Although most studies showed the usefulness of vitamin B6 in inflammatory conditions, apparently, our results could not confirm this therapeutic effect.[23] Even some investigations have shown that vitamin B6 deficiency would be evident in patients with IBD.[27] It seems that the results of our study are closer to the findings of Benight *et al*. (2011),[28] who reported that pyridoxine deficiency was protective against the colitis model caused by dextran sodium sulfate (DSS). It should be noted that in this study, vitamin B6 significantly reduced MPO and MDA variables in the target tissue. It should be mentioned that in this study, vitamin B6 was able to reduce the level of MDA and MPO variables in the colon tissue. It is likely that these effects were not enough to improve the manifestations of colitis in this study.

Two groups treated with combination of four vitamins (B1, B2, B6, and B12) at normal dose and with mega dose represented almost the same results. This indicated that meeting essential vitamin requirements was more important in protecting against colitis than providing vitamins with mega doses. This is in contrast with the claim that vitamins at mega dose could act as curative agents in some chronic inflammatory or cardiovascular diseases.[29] It seems that the consumption of vitamins in mega doses brings the risk of complications for the subjects as the lack of weight gain in animals treated with mega doses of four vitamins compared to the normal dose in this study.[29]

Unlike the results of macroscopic and microscopic evaluation, biochemical results showed that all of the treatment groups including the group which received mega dose of vitamin B6 were able to reduce MPO activity and MDA level significantly compared to the control group. This indicates that vitamin B6 and its active metabolite PLP have antioxidant and anti-inflammatory properties.[30] Although the exact antioxidant mechanism of vitamin B6 has not been confirmed yet, vitamin B6 may directly react with the peroxy radicals and thereby scavenge the radicals and inhibit lipid peroxidation.[30] A previous study executed by Taysi *et al*. (2005)[31] showed increased MDA level and glutathione reductase and peroxidase activity in the liver tissue of vitamin B6-deficient rats. However, MPO activity as a marker for neutrophil infiltration and tissue inflammation was attenuated by pyridoxine supplementation in rats with liver and lung injuries due to experimental sepsis.[32] Considering the beneficial effects of vitamin B6 on MPO and MDA parameters, it seems that other markers should also play a role in the oxidative and inflammatory processes of colitis.

Various inflammatory mediators, such as interleukin 1 beta (IL-1β), IL-6, TNF-α, and nitric oxide (NO), have been reported to be involved in the pathogenesis of UC.[24,25,27] In a study conducted on experimentally sepsis model, de Andrade *et al*. (2014)[6] showed that feeding rats with pellet chows enriched with thiamine pyrophosphate can reduce the level of many inflammatory markers in the blood, including IL-1β, IL-6, and TNF-α. In another study conducted by Afzal *et al*. (2017)[33] on the experimental model of hepatic cancer using diethyl nitrosamine, it was found that combination therapy of thiamine with ibuprofen played an important role in reducing inflammatory markers and tumor genesis factors. Also, many studies have confirmed the antioxidant and anti-inflammatory effects of vitamin B2. It seems that this vitamin independently or together with other antioxidants keeps the body's antioxidant capacity, especially in the glutathione redox cycle.[34] It has been shown that feeding rats with riboflavin-deficient diet caused a significant increase in lipid peroxidation marker (MDA) in various rat tissues.[35] In another study conducted by Wang *et al*., (2011)[36] it was shown that vitamin B2 (20 mg/kg/d) significantly reduced the level of MDA and lipid peroxidation in the myocardium of diabetic type 1 rats, while superoxide dismutase (SOD) capacity was increased. In another study conducted to investigate the anti-nociceptive and anti-inflammatory effect of riboflavin in animal models of hyperalgesia and inflammation, it was found that this vitamin can be effective in dose range of 6.25 to 150 mg/kg/day and competed with gabapentin.[10] Moreover, experiments in animals have shown that vitamins B1, B6, and B12 alone and in combination have anti-nociceptive activity against chemical- and heat-induced pain.[15,37] In a study conducted by Hosseinzadeh *et al*., (2012)[14] vitamin B12 in the dose range of 900–1800 μg/kg/d was able to improve acute and chronic inflammation caused by xylene and granuloma, respectively, in mice. In another study, which was conducted on the effect of vitamin B12 deficiency on colon tissue damage caused by DSS due to changes in intestinal microbiota, it was found that supplementing with this vitamin at high dose (200 μg/kg/d for 4 weeks) can improve intestinal microbiota and have a positive effect on colitis tissue lesions.[38] Therefore, another possibility to explain the effectiveness of group B vitamins in colitis, especially in high doses, could be their ability to improve and modify the intestinal microbiota, which needs further investigation.

Conclusions

It is concluded that four selected B vitamins in this study, except vitamin B6 even at mega dose, could improve experimental colitis in all the studied markers. Although vitamin B6 was not effective in improving microscopic

and macroscopic markers, it reduced some oxidative and inflammatory markers in the colon tissue, which can be considered positive. Therefore, it is recommended to measure other effective markers, such as cytokines and other inflammatory mediators in additional detailed studies.

Acknowledgements

The authors are grateful to the research vice-chancellor of the Isfahan University of Medical Sciences for the financial support of this project no. 3400476.

Financial support and sponsorship

The authors are grateful to the research vice-chancellor of the Isfahan University of Medical Sciences for the financial support of this project no. 3400476.

Conflicts of interest

There are no conflicts of interest.

Received: 26 Aug 23 **Accepted:** 20 Feb 24
Published: 28 Nov 24

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