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The effective role of vitamin D and omega-3 on rats with induced Crohn's disease

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***Abstract*---**Crohn's disease is an irreversible inflammatory disease, mainly affecting the gastrointestinal tract**.** The aim of present study was to investigate the effect of dietary vitamin D and omega 3 supplementation on rats with induced Crohn's disease. Twenty – four adult male albino rats weighing (160 gm) were divided into 4 groups. The 1st group (n=6) was fed on the basal diet. The 2nd to 4th of rats (n=18) were injected with (10 mg/kg for 3 days) of indomethacin at the end of the experiment to induce Crohn's disease. Groups (3 and 4) were fed on basal diet and given orally 1000/IU/kg/ b.w. of vitamin D and 600 mg/kg/b.w. of omega 3 respectively. At the end of the experimental period (8 weeks), rats were scarified. Biochemical analysis of the tested parameters was determined. The Results indicated that indomethacin Crohn's rats showed a significant (P< 0.05) decrease in serum albumin, serum Catalase and serum Glutathione peroxidase compared to the negative control one, while serum liver function, MDA, CRP, and immunity proteins were significantly increased. It was also observed that supplementation with vitamin D and/or omega 3 at the tested level significantly improved BWG%, liver functions, immunoglobulins, and antioxidant activity of Crohn's rats as compared to the positive control group. The results showed that rats with Crohn's disease and given vitamin D had significant increase in serum vitamin D concentration compared to positive control group. Moreover, an improved intestinal segment of ileum tissue was recorded in group given omega 3 as well. It could be concluded that omega 3 supplementation alleviating the side effect of Crohn's patients and had beneficial effects on Crohn's patients.

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***Keywords*---**Crohn's disease, inflammatory bowel diseases, vitamin D, omega3, rats.

Introduction

Crohn's disease (CD) is the main component of inflammatory bowel disease affecting the gastrointestinal tract, including abdominal pain, fever, and clinical signs of bowel obstruction or diarrhea with passage of blood or mucus, or both [1]. Heredity and a malfunctioning immune system, likely play a role in its development of Crohn's disease [2]. In Mediterranean countries, the prevalence of ulcerative colitis was estimated at 5/100000 in urban area [3]. Diets high in animal fat especially saturated animal fats and low in fruits and vegetables as well as vitamin D deficiency are the most common pattern associated with an increased risk of Irritable bowel diseases (IBD) [4].

Omega-3 fatty acids have been investigated for their anti-inflammatory properties as an alternative to traditional care. Fish oil preparation that limits the side effects of traditional fish oil therapy shows promise as an adjunctive treatment for Crohn’s disease [5]. Omega 3 fatty acids reduce intestinal inflammation, induce, and maintain clinical remission in Ulcerative colitis (UC) patients, and reduce of proinflammatory cytokines, and increase the quality of life of CD patients. Many studies have shown the beneficial effects of ω3 as adjunctive in the treatment or prevention of UC or CD [6]. Omega-3 fatty acids, abundant in fish oils, can relieve inflammation in the digestive tracts of people with Crohn's disease [7].

The benefits of supplementation with vitamin D IBD patients are still unclear, and improved vitamin D status may help to prevent the onset of IBD as well as ameliorating disease severity. Beneficial effects of vitamin D in IBD are supported by pre-clinical studies, notably with mouse models, where the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25-(OH)2D) has been shown to regulate gastrointestinal microbiota function, and promote anti-inflammatory, tolerogenic immune responses [8]. Due to the lack of studies handling the effect of diet contents and the risk of irritable bowel disease, this study was conducted.

Materials and Methods

Materials

Casein, vitamins, minerals, L cystine and choline bitartrate were obtained from El-Gomhoria Company, Cairo, Egypt. Capsules of Cholecalciferol (vitamin D3) at concentration of 1000 IU and Omega -3 were obtained from Delmar & Attallah Pharmacy, Cairo, Egypt. Starch, corn oil and sucrose were obtained from the local market. Kits for blood analysis were purchased from local distributer of (Sigma Chemical), Cairo, Egypt.

Methods

Induction of Crohn's disease (CD)

Rats were injected with indomethacin (10 mg/kg/b.w subcutaneous for 3 days) according to [9] at the beginning and at the end of the experiment. Tissue samples from ileum segments were collected and fixed in 10% neutral buffered formalin solution for histopathological examination according to [10] to insure the induction of Crohn's disease.

Experimental Animal Design

Twenty-Four adult male Albino rats were housed in well-aerated cages under hygienic conditions and fed on basal diet for one week for adaptation. The diet was formulated according to [11]. After this week, rats were assigned to the diet supplementation either with vitamin D and/or omega 3. Therefore, the tested groups were divided as follows: - Group (1): normal rats (negative control group) was fed on basal diet during all the experimental periods. Group (2): positive control rats were fed on basal diet and induction of disease pre and after the experimental period ended. Groups (3 and 4): As group 2 and were fed on basal diet and given orally (1000/IU/kg/ b.w. of vitamin D, 600 mg/kg/b.w. of omega 3), respectively.

During the experimental period, water and diet were introduced under hygienic conditions. At the end of the feeding period (8 weeks), rats were fasted over night before scarifying. Two blood samples were collected; one sample was centrifuged for serum collection for biochemical analysis, while the second (whole blood) sample was used for hematological parameters determination. This experiment was carried out at the animal house of Home Economics Faculty, Helwan University, Cairo - Egypt.

Biological Evaluation

Feed intake (FI), feed efficiency ratio (FER) and body weight gain percent (BWG%) were determined according to [12] using the following equation:

$$BWG\% = \frac{Final\ body\ weight(g) - initial\ bbody\ weight\ (g)}{initial\ body\ weight\ (g)} \times 100$$

$$\text{FER} = \frac{Body\ weight\ gain}{Feed\ intake}$$

Biochemical Analysis

Serum Immune globulin G (IgG) and globulin M (IgM) were determined according to [13,14], respectively. Serum Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) were determined by immunosorbent assay according to the method described by [15]. C- Reactive protein by [16]. Serum albumin as according to [17]. Serum catalase and glutathione peroxidase (GPx) were determined according to [18] while, serum malondialdehyde (MDA) was

determined according to [19]. Whole blood was used to determine red blood cells (RBCs), Hemoglobin (Hb), White blood cells (WBC) according to [20].

Statistical Analysis

The results are expressed as means ± Standard Error (SE). The obtained results are analyzed according to SPSS program, Version (20). ANOVA tests are used to compare results among groups and $P<0.05$ considered significant [21].

Results

Results illustrated in Table (1) show the effect of diets supplemented with vitamin D or omega 3 on body weight, feed intake and feed efficiency ratio of Crohn's rats. There were no significant changes in the initial body weight of all rats. The final body weight (FBW) and BWG% were significantly ($P< 0.05$) lowered in the positive control group as compared to the control negative one. Diet supplemented with either vitamin D or omega 3 significantly ($P<0.05$) increased the FBW, BWG% as compared to the positive control group. It was also observed that the highest FBW and BWG% were recorded at the group that given orally on omega 3. Moreover, the mean FI was increased at all different treated groups compared to the positive control group.

It was also observed that there were significant differences in FER among all the treated groups as compared to the positive control group. Then were no significant changes of FER between the groups given vitamin D or omega 3.

Table1: Effect of diets supplemented with Vitamin D and omega 3 on body weight, feed intake and feed efficiency ratio of Crohn's rats

Parameters Groups	IBW(g)	FBW(g)	BWG%	FI (g/day/rat)	FER
Control (-ve)	160.83±2.00^{a}	194.17±3.07^{c}	20.87±2.90^{b}	16.70	0.033±0.04^{b}
Control (+ve)	166.16±1.57^{a}	185.16±1.97^{d}	11.52±2.08^{c}	15.00	0.021±0.03^{c}
Vit D	166.00±1.86^{a}	204.50 ±3.01^{b}	23.24±1.97^{ab}	15.60	0.041±0.03^{ab}
Omega3	165.50±1.78^{a}	213.10±2.95^{a}	28.86±2.16^{a}	16.00	0.050±0.04^{a}

*Values are expressed as means ± SE.
*Values at the same column with different letters are significantly different at $P<0.05$.

Regarding to liver functions in Table (2), there were significant ($P<0.05$) increase in serum ALT and AST levels of the positive control group (rats with Crohn's disease) as compared to the negative control group. Feeding rats diets supplemented with vitamin D or omega 3 caused a significant ($P<0.05$) decrease in serum AST and ALT as compared to the positive control group. It was clear

that, there was significant (P<0.05) difference in serum ALT concentration between the tested groups. While there was no significant difference in serum AST level among the treated groups. The highest improvements for liver functions were observed at the group of rats that given on omega 3. Thus, the obtained results showed that ingestion with vitamin D or omega 3 improved serum liver functions concentration of Crohn's rats.

Table (2): Effect of diets supplemented with Vitamin D or omega 3 on serum liver functions in rats with Crohn's rats

Parameters Groups	AST(U/L)	ALT(U/L)
Control (-ve)	83.50±1.25^{c}	6.83±0.79^{d}
Control (+ve)	293.83±1.35^{a}	44.16±1.16^{a}
Vit D	284.23±1.07^{b}	40.50±1.17^{b}
Omega 3	282.34±0.94^{b}	36.33±1.28^{c}

*Values are expressed as means ± SE.
*Values at the same column with different letters are significantly different at P<0.05.

Effect of diets supplemented with omega 3 or vitamin D on serum albumin of Crohn's rats is shown in Table (3). There was a significant (P<0.05) decrease in serum albumin levels for the positive control group as compared to the control negative group. However, serum albumin levels were significantly (P<0.05) increased among all treated groups as compared to the positive control group. Moreover, there was a significant difference in serum albumin between the group given vitamin D or omega3.

The highest increase in serum albumin concentration was observed at the group of rats fed on basal diet supplemented with omega 3 .

Table (3): Effect of diets supplemented with vitamin D or omega 3 on serum albumin in rats with Crohn's rats

Parameters Groups	Albumin (gm / dL)
Control (-ve)	33.16±1.07^{a}
Control (+ve)	0.33±0.02^{d}
Vit D	5.60±0.10^{c}
Omega 3	13.75±0.21^{b}

*Values are expressed as means ± SE.
*Values at the same column with different letters are significantly different at P<0.05.

The results in Table (4) illustrated the effect of diets supplemented with vitamin D or omega 3 on hematological parameters of Crohn's rats. The positive control rats (Crohn's rats) had a significant (P<0.05) increase in the mean value of WBC and significant decrease in RBC and Hb concentrations as compared to the negative control group. However, the diet supplemented with vitamin D or omega 3 caused significant decrease in the concentration of WBC compared to the positive control

group, however, the levels of RBC and Hb are significantly increased as compared to the +ve control group. there were no significant differences in WBC , RBC and Hb between the treated groups.

Table (4): Effect of diets supplemented with vitamin D and omega 3 on haematological parameters of Crohn's rats

parameters Groups	WBC ($\times 10^3$/cmm)	RBC ($\times 10^6$/cmm)	Hb (g/dl)
Control (-ve)	4.98±0.18^c	5.38±0.18^a	13.01±0.14^a
Control (+ve)	8.41±0.15^a	3.61±0.20^c	9.93±0.27^c
Vit D	6.85±0.17^b	4.25±0.13^b	10.55±0.09^b
Omega 3	6.73±0.52^b	4.41±0.27^b	10.71±0.17^b

* Values are expressed as means ± SE.
*Values at the same column with different letters are significantly different at P<0.05.

The effect of diets supplemented with vitamin D and/or omega 3 on the inflammatory response (C-RP, CAT, GPx and MDA) of Crohn's rats are shown in Table (5). There were a significant (P<0.05) increase in serum C-RP, and MDA while, serum GPx and CAT levels were significantly (P<0.05) decreased at the positive control group as compared to the negative control group. There was a significant difference in CRP, MDA, GPX, CAT between the treated groups. Thus, our study showed that ingestion of diets supplemented with vitamin D and omega 3 improved serum C-RP, MDA, GPX and CAT of Crohn's rats.

Table (5): Effect of diets supplemented with vitamin D or omega 3 on serum antioxidant enzymes of Crohn's rats

Parameters Groups	CRP (mg/dL)	MDA (nmole/ml)	GPX (µmole /ml)	CAT (ng /ml)
Control (-ve)	20.33±1.38^d	44.00±1.06^d	293.33±1.45^a	287.00±2.23^a
Control (+ve)	62.35±2.49^a	292.16±2.56^a	75.33±2.99^d	72.00±0.96^d
Vit D	52.12±1.94^b	280.00±1.15^b	258.83±2.37^c	264.00±1.21^c
Omega 3	46.50±1.87^c	256.83±2.65^c	267.67±1.14^b	272.33±1.22^b

* Values are expressed as means ± SE.
*Values at the same column with different letters are significantly different at P<0.05.

The results in Table (6) illustrated the effect of diets supplemented with vitamin D or omega 3 on the immune functions (IgG and IgM) of Crohn's rats. Serum IgG and IgM were significantly decreased for the +ve control group as compared to the -ve control group. When rats fed on basal diet and given orally vitamin D caused no significant change in serum IgG while, omega 3 caused a significant increase in serum IgG as compared to the positive control group. Moreover, there was no significant changes in IgG between the groups given Vit D or Omega 3. The most improvement in serum IgG and IgM were recorded at the group given omega 3.

Table (6): effect of diets supplemented with vitamin D and/or omega 3 on the immunity proteins of serum IgG and IgM.

Parameters Groups	IgG (IU)	IgM (IU)
Control (-ve)	103.86±1.73^{a}	233.05±1.12^{a}
Control (+ve)	83.50±1.17b^{c}	84.16±1.53^{e}
Vit D	82.83±2.67^{bc}	213.83±3.89^{c}
Omega 3	86.33±2.60^{b}	221.50±1.38^{b}

* Values are expressed as means ± SE.
*Values at the same column with different letters are significantly different at P<0.05

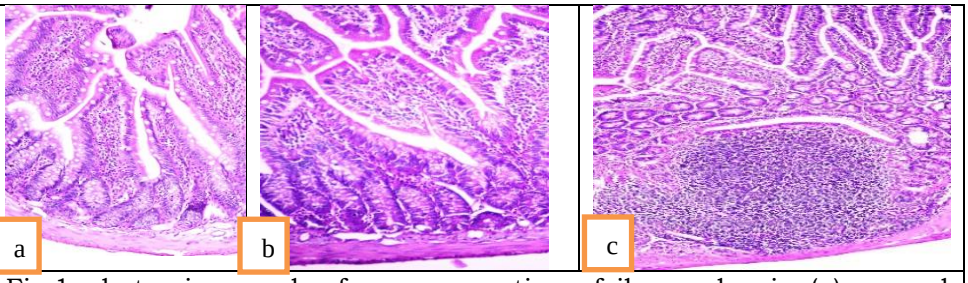
The results in Table (7) showed the concentrations of serum Vitamin D of Crohn's rats. There was a significant (P<0.05) decrease in serum vit. D levels for the positive control group compared to the normal negative control group. However, serum vit D levels were significantly (P<0.05) increased for the group fed on basal diet and given vitamin D as compared to the positive control group. On the other hand, it was observed that, omega 3 supplementation caused no significant difference in the serum concentration of vitamin D as compared to the positive control group. The highest increase in serum vitamin D concentration was observed at the group of rats given vitamin D.

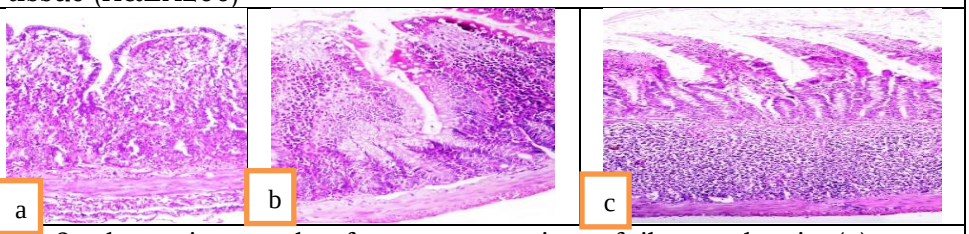
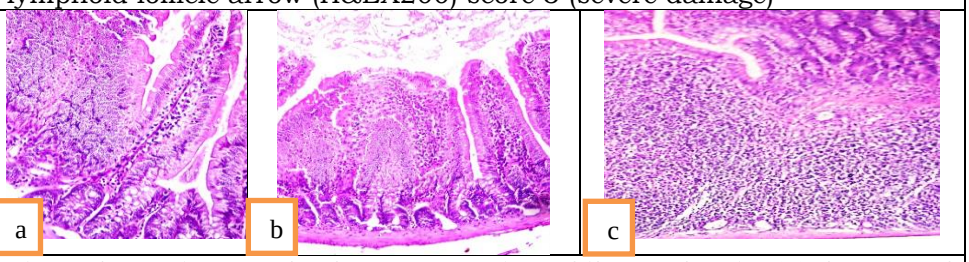
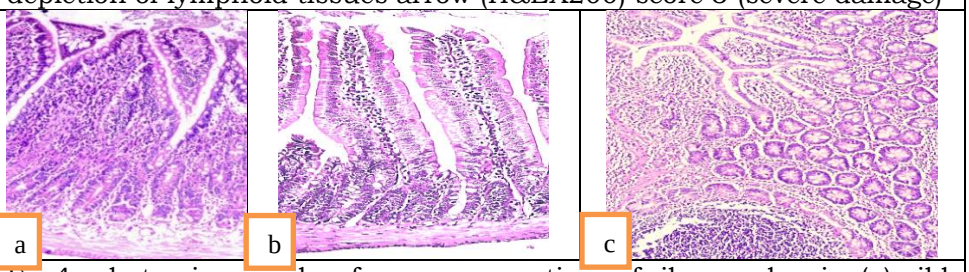
Table (7): effect of diets supplemented with vitamin D or omega 3 on the concentrations of serum Vitamin D of Crohn's rats

Parameters Groups	Vitamin D (nmol/L)
Control (-ve)	20.37±0.21^{a}
Control (+ve)	4.29±0.02^{c}
Vit D	6.08±0.10^{b}
Omega 3	4.66±0.04^{c}

* Values are expressed as means ± SE.
*Values at the same column with different letters are significantly different at P<0.05.

Histopathological Examination

Control (-ve) group	 Fig.1 photomicrograph of a cross section of ileum showing(a) normal histological structure of intestinal mucosa arrow (b) Numerous numbers of goblet cells in-between the columnar cells arrow head with

	intact crypts arrow (c) Normal histological architecture of lymphoid tissue (H&EX200)
Control (+ve) group	 Fig.2 photomicrograph of a cross section of ileum showing(a)severe damage of intestinal mucosa with loss of goblet cells arrow (b) Haphazard crypt organization and numerous numbers of inflammatory cells infiltration arrow (c) shorting of intestinal villi and depletion of lymphoid follicle arrow (H&EX200) score 3 (severe damage)
Vit D group	 Fig.3 photomicrograph of a cross section of ileum showing(a) damage of intestinal villi arrow (b) massive inflammatory cells infiltration and with distortion of intestinal crypt arrow (c) necrosis of intestinal glands with depletion of lymphoid tissues arrow (H&EX200) score 3 (severe damage)
Omega 3 group	 Fig.4 photomicrograph of a cross section of ileum showing(a)mild epithelial shedding and hyperactivity of goblet cells arrow (b) normal intestinal crypt and few inflammatory cells infiltration arrow (c) intact glandular epithelial lining and lymphoid tissues arrow (H&EX200) score 0 (marked improvement)

Discussion

Crohn's disease, is one of the most frequent forms of inflammatory disease worldwide, is characterized by the formation of strictures, fistulas, ulcers, and granulomas in the mucosa. The clinical manifestations of CD can include diarrhea or bloody diarrhea, malnutrition, abdominal pain, and weight loss [22]. The obtained results indicated that diet supplemented with omega 3 significantly ($P<0.05$) improved the mean levels of serum IgG and IgM in Crohn's rats.

This finding is consistent with the work of [23] who reported that while mucosal IgG responses are common to both UC and CD, significant differences are observed in the targets and inflammatory characteristics of these responses that may impact disease susceptibility. The barrier function of vitamin D is also linked to its impact on the gastrointestinal microbiota, with serum 25-OH-D status in humans being correlated with changes in gastrointestinal bacterial genera associated with inflammatory immune responses.

The results of the current study revealed an improvement in serum C-RP, MDA, GPX and CAT the group of rats given omega 3. These results are confirmed by the findings of [24] who found increase in MDA and hydroperoxide levels which are indicator for the tissue injury. Catalase and Glutathione were reduced significantly in the colonic tissues of the acetic acid induced ulcerative colitis in rats. Catalase is one of the crucial antioxidant enzymes that mitigates oxidative stress to a considerable extent by destroying cellular hydrogen peroxide to produce water and oxygen [25].

There is a significant decrease in serum of albumin levels and almost 50% of the Crohn's disease patients had one or more pre-operative abnormal values of Hb, albumin and CRP. These results are agreed with the obtained results [26]. The results of the current study showed an improvement in the hematological parameters at the group of rats given omega 3. These findings are harmony with the results of [27] who reported an elevated WBC count in patients with active IBD and does not necessarily mean infection. High leukocyte count is also common in patients taking steroids due to drug-induced mobilization of marginated neutrophils.

The results of the current study showed a significant decrease in RBC and Hb concentrations as compared to the normal rats. These results are agreed with [28] who found that Patients with IBD are commonly found to have iron deficiency anemia, secondary to chronic blood loss, and impaired iron absorption due to tissue inflammation. Patients with iron deficiency may not always manifest with signs and symptoms; so, hemoglobin levels in patients with IBD must be regularly monitored for earlier detection of anemia.

Patients with CD sometimes show transient abnormalities in liver function test (LFT) results. Although primary sclerosing cholangitis and drugs for the treatment of CD are common causes of abnormal liver function findings, patients with CD may also have cryptogenic liver injury [29]. The results of the current study showed a significant ($P<0.05$) decrease in serum vit. D levels for the positive control group as compared to the normal control group. This result is confirmed by the findings of [30] reported that Crohn's disease prevalence increases with increasing latitude. Because most vitamin D comes from sunlight exposure and murine models of intestinal inflammation have demonstrated beneficial effects of 1,25-(OH)2 vitamin D treatment, we hypothesized that Crohn's disease activity is associated with low vitamin D levels.

The Results indicated that rats with Crohn's and given vitamin D had significant increase in serum vitamin D concentration as compared to the positive control group these results are agree with [8] reported that vitamin D has a protective role

in IBD by maintaining the intestinal epithelial barrier through immune interactions that favorably affect the gut microbiome and influence the gut's immune system by modulating the innate and adaptive immune systems. Vitamin D deficiency as a result of IBD has been increasingly recognized to contribute to a dysregulated intestinal immune response. Concerning the photomicrograph of a cross section of ileum, the result of current study indicates that diets supplementation with omega 3 improved the epithelial lining and lymphoid tissue whilst the group with fed on just vitamin D showed necrosis of intestinal glands and depletion of lymphoid tissue of score 3 (severe damage).

Given vitamin D (1200IU) to 108 patients with CD once daily for 12 months, might be effective in CD that reduced the risk of relapse from 29% to 13% [31]. Whilst some studies agree with current study. [5] found that fish oil preparation that limits the side effects of traditional fish oil therapy shows promise as an adjunctive treatment for Crohn's disease. Omega 3 fatty acids reduce intestinal inflammation, induce and maintain clinical remission in UC patients, and are related with the reduction of pro inflammatory cytokines, decrease disease activity and increase the quality of life of CD patients[4,32]. In conclusion, since results of our study showed that omega 3 given orally had beneficial effects on Crohn's rats, so, it could be recommended that omega 3 supplementation might be worthy protecting for Crohn's disease patients.

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