

## **EFFICACY OF DIET SUPPLEMENTATION WITH 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. Thirty adult male albino rats weighing (160 gm) were divided into 5 groups. The 1<sup>st</sup> group (n=6) was fed on the basal diet. The 2<sup>nd</sup> to 5<sup>th</sup> of rats ( n=24) were injected with (10 mg/kg for 3 days) of indomethacin to induce Crohn's disease. Groups (3 to 5) were fed on basal diet and given orally 1000/IU/kg/bw of vitamin D, 600 mg/kg/bw of omega 3 and combination of 1000/IU/kg/ bw of vitamin D and 600 mg/kg/bw of omega 3, respectively. At the end of the experimental period (8 weeks), rats were scarified. Biochemical analysis of the tested parameters was carried out.

Results indicated that indomethacin Crohn's rats showed a significant ( $P < 0.05$ ) decrease in serum albumin, catalase and glutathione peroxidase compared to the negative control one, while serum liver function , MDA ,CRP, and immunity proteins were significantly increased.

Results showed that Crohn's rats given vitamin D had significant increased serum vitamin D concentration compared to positive control group .On the other hand, it was observed that, the diet supplemented with omega 3 caused no significant difference in the serum concentration of vitamin D compared to the positive control group .The highest increase in serum vitamin D concentration was observed at the group of rats fed on omega 3 and vitamin D.

It was also observed that supplementation of rats with vitamin D and/or omega 3 at the tested level improved BWG%, liver functions, immunoglobulins and antioxidant activity of Crohn's rats as compared to the positive control group. Also an improved of intestinal segment of ileum tissue was recorded as well. It could be concluded that combination with vitamin D and omega 3 supplementation alleviating the side effect of Crohn's rats. So, these materials may be benefit for Crohn's patients.

**Key Words :-** Crohn's disease , Inflammatory bowel diseases , Vitamin D , Omega 3 ,

## 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 (**Baumgart and Sandborn, 2012**).

Heredity and a malfunctioning immune system, likely play a role in its development of Crohn's disease (**Shanahan, 2002**). In Mediterranean countries, the prevalence of ulcerative colitis was estimated at 5/100000 in urban area (**Ahuja and Tandon, 2010**).

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) (**Lewis and Abreu, 2017**).

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 showed promise as an adjunctive treatment for Crohns disease **MacDonald, (2006)**.

**Shafiee, et al., (2021)** showed that  $\omega$ 3 fatty acids reduce intestinal inflammation, induce and maintain clinical remission in ulcerative colitis patients, reduce of proinflammatory cytokines, and increase the quality of life of CD patients. Many studies have shown the beneficial effects of  $\omega$ 3 as adjunctive in the treatment or prevention of UC or CD.

**Turner, et al., (2011)** suggested that omega-3 fatty acids, abundant in fish oils, can relieve inflammation in the digestive tracts of people with Crohn's disease.

Vitamin D has been linked to human health benefits that extend far beyond its established actions on calcium homeostasis and bone metabolism. One of the most well studied facets of extra-skeletal vitamin D is its activity as an immuno-modulator, in particular its potent anti-inflammatory effects. As a consequence, vitamin D deficiency has been associated with inflammatory diseases including inflammatory bowel disease (IBD). Low serum levels of the major circulating form of vitamin D, 25-hydroxyvitamin D (25-OH-D) are significantly more prevalent in patients with IBD, particularly in the winter and spring months when UV-induced synthesis of vitamin D is lower. Dietary malabsorption of vitamin D may also contribute to low serum 25(OH)D in IBD. The benefits of supplementation with vitamin D for 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)<sub>2</sub>D) has been shown to regulate gastrointestinal microbiota function, and promote anti-inflammatory, tolerogenic immune responses (**Fletcher *et al.*, 2019**).

Due to the lack studies handling the effect of diet contents and the risk of irritable bowel disease, this study was conducted.

## MATERIALS AND METHODS

### A. Materials

Casein, vitamins, minerals, L.cystine and choline bitartrate were obtained from El-Gomhoria Company, Cairo, Egypt . Capsules of Cholecalciferol (vitamin D<sub>3</sub>) 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 distributor of (Sigma Chemical), Cairo, Egypt.

Animals: A total number of thirty adult male albino rats (Sprague -Dawley strain) weighing about 160 gm was purchased from the Animal House of Helwan Farm, Helwan, Egypt.

### B. Methods:

#### 1. Induction of Crohn's disease (CD):

Rats was injected with indomethacin (10 mg/kg subcutaneous for 3 days) according to **Simon *et al.*, (2017)**. Tissue samples from ileum segments were collected and fixed in 10% neutral buffered formalin solution for histopathological examination according to **Bancroft and Gamble, (2013)**.

#### 2. Experimental Animal Design:

Thirty 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 **Reeves *et al.*, (1993)**. 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) were fed on basal diet during all the experimental period. **Group (2):** rats were fed on basal diet and injected with Indomethacin. **Groups (3 to 5):** as group 2 and were fed on basal diet and given orally 1000/IU/kg/bw of vitamin D, 600 mg/kg/bw of omega 3 fatty and combination of 1000/IU/kg/bw of vitamin D and 600 mg/kg/bw of omega 3 fatty, 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.

- 3. Biological Evaluation:** Feed intake (FI), feed efficiency ratio (FER) and body weight gain per cent (BWG%) were determined according to **Bakr and Header, (2014)** using the following equation:

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

$$\text{FER} = \text{Body weight gain (g)} / \text{Feed intake (g)}$$

- 4. Biochemical Analysis:** Serum Immune globulin G (IgG) and globulin M (IgM) were determined according to **McEwan et al., (1970)** and **Liesenfeld et al., (1997)**, respectively. Serum Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) were determined by immune sorbent assay according to the method described by **Thomas, (1998)**. C- Reactive protein was determined as described by **Kao et al., (2006)**. Serum albumin was demined according to **Lippi et al., (2007)**. Serum catalase and glutathione peroxidase (GPx) were determined according to **Goth, (1991)**. While serum malondialdehyde (MDA) was determined according to **Ermis et al., (2004)**. Whole blood was used to determine red blood cells (RBCs), Hemoglobin (Hb), White blood cells (WBC) according to **Reilly et al., (1997)**.

- 5. Statistical Analysis:**

The results are expressed as means  $\pm$  Standard Error (SE). The obtained results were analyzed according to SPSS program, Version (20). ANOVA test was used to compare results among groups and  $P < 0.05$  considered significant (**Snedecor and Cochran, 1980**).

## 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 body weight gain per cent (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 or their combination 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 fed on combination with vitamin D and omega 3. Moreover, the mean feed intake 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.

**Table (1): Effect of diets supplemented with vitamin D and omega 3 on body weight, feed intake and feed efficiency ratio of Crohn's rats**

Parameter Group	IBW(g)	FBW(g)	BWG%	FI (g/day/rat)	FER
Control (-ve)	160.83±2.00 <sup>a</sup>	195.00±2.52 <sup>b</sup>	21.37±2.51 <sup>ab</sup>	16.70	0.034±0.03 <sup>ab</sup>
Control (+ve)	164.63±3.34 <sup>a</sup>	178.83±2.03 <sup>c</sup>	8.73±2.75 <sup>c</sup>	14.50	0.016±0.04 <sup>c</sup>
Vit D	163.33±1.97 <sup>a</sup>	194.33±2.48 <sup>b</sup>	16.57±2.15 <sup>b</sup>	15.20	0.046±0.02 <sup>a</sup>
Omega 3	162.00±3.19 <sup>a</sup>	202.66±2.23 <sup>a</sup>	25.39±3.20 <sup>a</sup>	15.00	0.045±0.04 <sup>a</sup>
Combination	166.80±1.92 <sup>a</sup>	205.33±1.85 <sup>a</sup>	25.78±1.52 <sup>a</sup>	16.30	0.028±0.03 <sup>b</sup>

\*Values are expressed as means ± SE.

\*Values at the same column with different letters are significantly different at P<0.05

\*IBW = Initial body weight, FBW= Final body weight, BWG% = Body weight gain per cent, FI = Feed intake, FER=Feed efficiency ratio.

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) compared with the negative control group (normal rats). Feeding rats diets supplemented with vitamin D, omega 3 and in combination with vitamin D and omega 3 caused a significant (P<0.05) decrease in serum AST and ALT compared to the positive control group. It was clear that, there was significant difference in serum ALT concentration between the tested groups with either vitamin D or omega 3. Moreover, there was significant difference in serum AST level among all the treated groups. The highest improvements for liver functions were observed at the group of rats that fed on combination of vitamin D and omega 3. Thus, the obtained results showed that ingestion with vitamin D and omega 3 improved serum liver functions concentration of Crohn's rats.

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

Parameter Group	AST(U/L)	ALT(U/L)
Control (-ve)	83.66±1.17 <sup>c</sup>	6.96±0.74 <sup>d</sup>
Control (+ve)	284.38±1.41 <sup>a</sup>	34.16±1.16 <sup>a</sup>
Vit D	275.90±1.48 <sup>b</sup>	27.66±1.25 <sup>b</sup>
Omega 3	270.25±2.16 <sup>c</sup>	24.50±0.76 <sup>c</sup>
Combination	262.06±2.68 <sup>d</sup>	21.76±0.82 <sup>c</sup>

\*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 and/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. Results indicated that there was significant difference in albumin level among all the treated groups. The highest increase in serum albumin concentration was observed at the group of rats fed on combination with omega 3 and vitamin D. Thus, the obtained data recorded that ingestion of vitamin D and omega 3 improved serum albumin concentration of Crohn's rats.

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

Group	Parameter	Albumin (gm / dL)
Control (-ve)		33.16±1.07 <sup>a</sup>
Control (+ve)		0.43±0.01 <sup>e</sup>
Vit D		5.49±0.07 <sup>d</sup>
Omega 3		12.50±0.01 <sup>c</sup>
Combination		17.55±0.01 <sup>b</sup>

\*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 and/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 or in combination 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. It was clear that, there are no significant changes in the level of RBC and Hb among all treated groups. The highest improvement in WBC, RBC and Hb concentrations were observed in the group of rats fed on basal diet supplemented with combination of vitamin D and omega 3.

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

Group	Parameter	WBC( $\times 10^3$ /cmm)	RBC( $\times 10^6$ /cmm)	Hb(g/dl)
-ve		4.98±0.23 <sup>d</sup>	5.38±0.20 <sup>a</sup>	13.01±0.13 <sup>a</sup>
+ve		8.10±0.28 <sup>a</sup>	3.75±0.18 <sup>c</sup>	9.88±0.34 <sup>e</sup>
Vit D		6.71±0.23 <sup>b</sup>	4.60±0.13 <sup>b</sup>	10.95±0.19 <sup>b</sup>
Omega		5.96±0.13 <sup>bc</sup>	4.53±0.20 <sup>b</sup>	10.95±0.24 <sup>b</sup>
Combination		5.75±0.37 <sup>cd</sup>	5.13±0.29 <sup>a</sup>	11.30±0.19 <sup>b</sup>

\*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 is shown in **Table (5)**. There were a significant ( $P<0.05$ ) increase in serum C-RP, and MDA levels for the positive control group compared to the negative control group. However, serum , GPx and CAT levels were significant ( $P<0.05$ ) decreased in positive control group compared to the negative control group. The highest improvement in serum C-RP, MDA , , GPX and CAT was observed in the group of rats fed on combination vitamin D and omega 3. Thus, present 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 and omega 3 on serum C-RP, CAT, GPx and MDA of Crohn's rats**

Parameter Group	CRP (mg/dL)	MDA(nmole/ml)	GPX ( $\mu$ mole /ml)	CAT(ng /ml)
-ve	20.33 $\pm$ 1.38 <sup>d</sup>	44.00 $\pm$ 1.06 <sup>d</sup>	293.33 $\pm$ 1.45 <sup>a</sup>	264.00 $\pm$ 1.21 <sup>a</sup>
+ve	55.83 $\pm$ 1.30 <sup>a</sup>	283.66 $\pm$ 0.66 <sup>a</sup>	57.00 $\pm$ 1.06 <sup>c</sup>	84.83 $\pm$ 1.44 <sup>d</sup>
Vit D	47.31 $\pm$ 2.12 <sup>b</sup>	272.33 $\pm$ 1.20 <sup>b</sup>	261.00 $\pm$ 1.29 <sup>d</sup>	244.16 $\pm$ 1.30 <sup>c</sup>
Omega	43.00 $\pm$ 1.63 <sup>b</sup>	268.83 $\pm$ 2.67 <sup>b</sup>	274.83 $\pm$ 1.19 <sup>c</sup>	249.16 $\pm$ 2.86 <sup>b</sup>
Combination	36.67 $\pm$ 1.60 <sup>c</sup>	244.33 $\pm$ 1.94 <sup>c</sup>	282.16 $\pm$ 0.87 <sup>b</sup>	253.00 $\pm$ 1.03 <sup>b</sup>

\*Values are expressed as means  $\pm$  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 and/or omega 3 on the immune functions (IgG and IgM) of Crohn's rats. Serum IgG and IgM are significantly increased for the +ve control group as compared to the -ve control group. When rats fed on basal diet and given orally vitamin D and/or omega 3 caused significant decrease ( $P<0.05$ ) in serum IgG and IgM compared to the positive control group. There was a significant difference in serum IgM among the treated rats. However, there was non significant change in serum IgG for the group fed on basal diet and given omega 3 as compared to the group given vitamin D. Also, the same trend was observed between the groups given omega 3 and the group given combination of vitamin D and omega 3. The most improvement in serum IgG and IgM were recorded at the group given combination of vitamin D and omega 3.

**Table (6): Effect of diets supplemented with vitamin D and/or omega 3 on the immunity proteins of serum IgG and IgM of Crohn's rats.**

Parameter Group	IgG (IU)	IgM (IU)
-ve	103.86 $\pm$ 1.73 <sup>a</sup>	233.05 $\pm$ 1.12 <sup>a</sup>
+ve	83.50 $\pm$ 1.17 <sup>b</sup>	84.16 $\pm$ 1.53 <sup>c</sup>
Vit D	78.00 $\pm$ 1.26 <sup>c</sup>	206.50 $\pm$ 3.25 <sup>d</sup>
Omega	82.83 $\pm$ 2.67 <sup>bc</sup>	213.83 $\pm$ 3.89 <sup>c</sup>
Combination	86.33 $\pm$ 2.60 <sup>b</sup>	221.50 $\pm$ 1.38 <sup>b</sup>

\*Values are expressed as means  $\pm$  SE.

\*Values at the same column with different letters are significantly different at  $P<0.05$ .

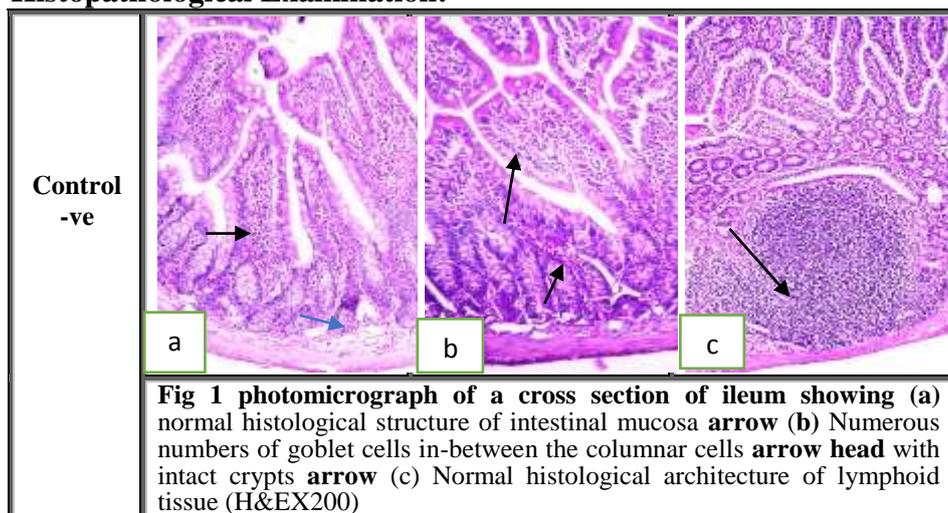
The results in **Table (7)** illustrated the effect of diets supplemented with vitamin D and/or omega 3 on the concentrations of Serum Vitamin D of Crohn's rats.

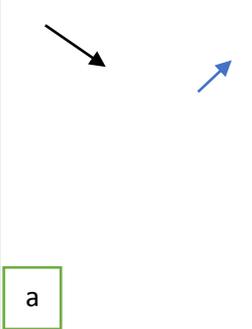
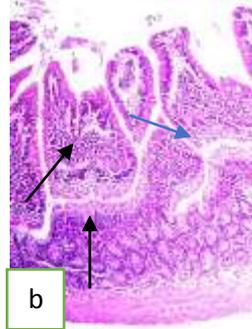
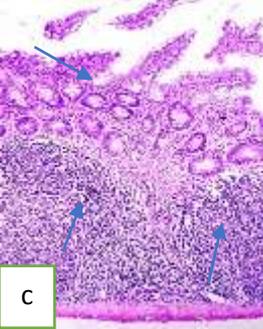
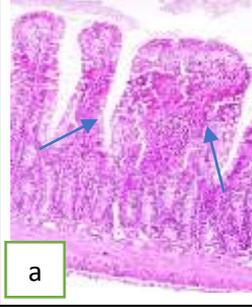
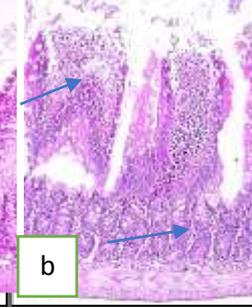
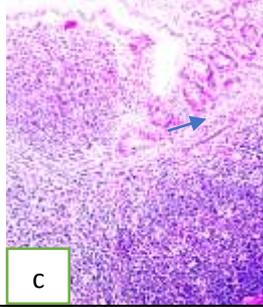
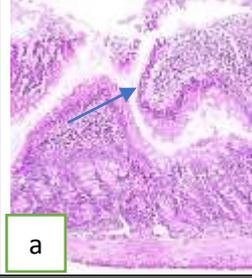
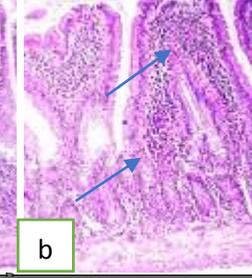
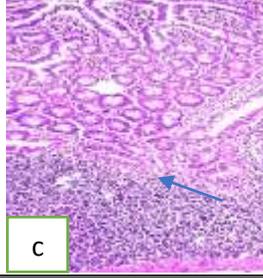
There was a significant ( $P < 0.05$ ) decrease in serum vit. D levels for the positive control group compared to the normal negative control group. However, serum vit.D levels was significantly ( $P < 0.05$ ) increased among all treated groups compared to the positive control group. Results indicated that rats with Crohn's and ingested vitamin D had significantly increase serum vitamin D concentration compared to positive control group .On the other hand it was observed that, the diet supplemented with omega 3 caused no significant difference in the serum concentration of vitamin D compared to the positive control group. The highest increase in serum vitamin D concentration was observed at the group of rats fed on omega 3 and vtamin D. Thus, current study showed that ingestion of vitamin D and omega 3 improved serum vitamin D concentration of crohn's rats.

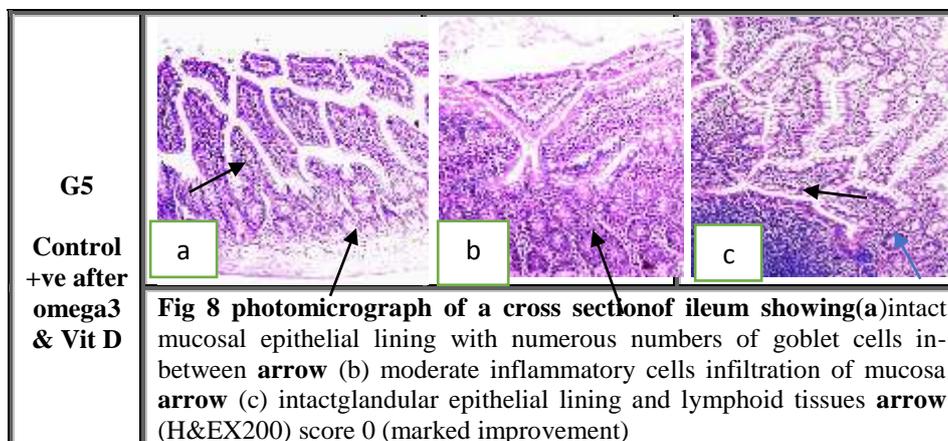
**Table (7): Effect of diets supplemented with vitamin D and/or omega 3 on the concentrations of serum vitamin D of Crohn's rats.**

Group	Parameter	Vitamin D (nmol/L)
-ve		20.37±0.21 <sup>a</sup>
+ve		5.63±0.07 <sup>d</sup>
Vit D		6.69±0.06 <sup>c</sup>
Omega		5.79±0.02 <sup>d</sup>
Combination		7.96±0.10 <sup>b</sup>

#### Histopathological Examination:



<p><b>G2</b> <b>Control +ve after</b></p>			
<p><b>Fig 2 photomicrograph of a cross section of ileum showing(a) desquamation of epithelial lining with blunted villus pattern arrow (b) inflammatory cells infiltration and distortion of intestinal crypts arrow (c) shorting of intestinal villi and depletion of lymphoid follicle arrow (H&amp;EX200) score 2 (mild damage)</b></p>			
<p><b>G3</b> <b>Control +ve after vit D</b></p>			
<p><b>Fig 3 photomicrograph of a cross section of ileum showing(a) severe damage of intestinal mucosa with distortion of intestinal crypts arrow (b) massive inflammatory cells infiltration in both mucosa and submucosa arrow (c) necrosis of intestinal glands and depletion of lymphoid tissues arrow (H&amp;EX200) .score 3 (severe damage)</b></p>			
<p><b>G4</b> <b>Control +ve after omega3</b></p>			
<p><b>Fig 4 photomicrograph of a cross section of ileum showing(a) mild epithelial shedding at the apices of some intestinal villi with marked loss of goblet cells arrow (b) moderate crypt distortion and heavy inflammatory cells infiltration arrow (c) moderate damage of glandular epithelial lining with apoptosis of lymphoid tissues arrow (H&amp;EX200). score 2 (moderate improvement)</b></p>			



### 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 (**Panaccione, 2013**).

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 **Castro-Dopico et al., (2020)** 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 a significant ( $P < 0.05$ ) increase in serum C-RP, and MDA levels. However, serum, GPx and CAT levels were significantly ( $P < 0.05$ ) decreased in positive control group compared to the negative control group. The highest improvement in serum C-RP, MDA, GPX and CAT was observed in the group of rats fed omega 3. These results are confirmed by the findings of **Hussein et al., (2019)** 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. **Nandi et al., (2019)** have demonstrated a 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. **Ghoneima et al., (2019)** found 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 agree with the obtained results.

The results of the current study showed that the highest improvement in the hematological parameters was observed at the group of rats fed on omega 3. These findings are in harmony with the results of (Cappello and Morreale, 2016) 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 that significant decrease in RBC and Hb concentrations as compared to normal rats. These results agree with Kaitha *et al.*, (2015) who found that patients with IBD are commonly found to have iron deficiency anemia (IDA) 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 (Furuta *et al.*, 2008).

The results of the present study, showed that there was a significant ( $P < 0.05$ ) decrease in serum vit. D levels for the positive control group compared to the normal control group. This result is confirmed by the findings of (Bartels *et al.*, 2013) who 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)<sub>2</sub> vitamin D treatment. We hypothesised 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 compared to the positive control group these results are in agreement with Fletcher *et al.*, (2019) who suggested that vitamin D has a probable role as an anti-inflammatory agent with potential activity against microbes via vitamin D receptors (VDRs) located in most nucleated cells. Vitamin D has a protective role in IBD by maintaining the intestinal epithelial barrier through immune interactions that favorably affect the gut microbiome. Furthermore, vitamin D has been noted to 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 deregulated intestinal immune response.

Concerning the photomicrograph of a cross section of ileum, the result of current study indicated 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). However, some studies contrast with current study .

**Bartels et al., (2013)** found that 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%.whilst some studies agree with current study. **MacDonald, (2006)** found that fish oil preparation that limits the side effects of traditional fish oil therapy shows promise as an adjunctive treatment for Crohns disease. **Brennan, et al., (2020) and Lewis and Abreu (2017)** showed that  $\omega$ 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.

In conclusion, since results of the present study showed that omega 3 given orally had beneficial effects on Crohn's rats, so, it could be recommended that omega 3 supplementation be worthy protecting for Crohn's disease patients.

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### فعالية استخدام مكملات فيتامين د وأوميغا 3 على الفئران

#### المصابة بمرض كرون

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مرض كرون هو مرض التهابي لا رجعة فيه ، ويؤثر بشكل رئيسي على الجهاز الهضمي. الهدف من هذه الدراسة هو معرفة تأثير مكملات فيتامين د وأوميغا 3 الغذائية على الفئران المصابة بداء كرون المستحث. تم تقسيم ثلاثين من ذكور الفئران البالغة وزنها 160 جم إلى 5 مجموعات. المجموعة الأولى (ن = 6) كانت تتغذى على النظام الغذائي الأساسي. تم حقن الثاني إلى الخامس من الفئران (ن = 24) بـ (10 مجم / كجم لمدة 3 أيام) من الإندوميثاسين لاستحداث مرض كرون. تم إعطاء المجموعات (من 3 إلى 5) على النظام الغذائي الأساسي وتم إعطاؤهم عن طريق الفم 1000 / وحدة دولية / كجم / وزن الفأر فيتامين د ، 600 مجم / كجم / وزن الفأر من أوميغا 3 ، ومزيج من 1000 / وحدة دولية / كجم / وزن الجسم من فيتامين د و 600 ملجم / كجم / وزن الجسم من أوميغا 3 على التوالي. في نهاية فترة التجربة (8 أسابيع) ثم تم قتل الفئران لعمل التحاليل البيوكيميائية .

أشارت النتائج الى ان الفئران المصابة بمرض كرون اظهرت انخفاض معنوي في الالبومين وكتاليز والجلوتاثيون بيروكسيديزمقارنة بالمجموعة الكنترول السالبة بينما زادت بشكل معنوي في وظائف الكبد والبروتينات المناعية MDA و CRP و

أظهرت النتائج أن الفئران المصابة بمرض كرون والتي تناولت فيتامين د زادت معنوياً في تركيز فيتامين د في الدم مقارنة بمجموعة الكنترول الموجبة ، ومن ناحية أخرى لوحظ أن النظام الغذائي المضاف إليه أوميغا 3 لا يسبب فرقاً معنوياً في تركيز فيتامين د في الدم مقارنة مع المجموعة الضابطة الموجبة . لوحظت أعلى زيادة في تركيز فيتامين (د) في مصل الدم في مجموعة الفئران التي تم تغذيتها على أوميغا 3 وفيتامين د.

ولوحظ أيضًا أن تناول مكملات فيتامين (د) و / أو أوميغا 3 أدى الى تحسن الوزن المكتسب للفئران ووظائف الكبد والجلوبيولين المناعى ونشاط مضادات الاكسدة للفئران المصابة بمرض كرون كما تم تسجيل تحسن فى الجزء المعوى من نسيج اللفائفى مما أدى الى استنتاج ان الجمع بين بين فيتامين د ومكملات أوميغا 3 يخفف من الآثار الجانبية لمرضى كرون ، لذلك كان لهذه المواد آثار مفيدة على مرضى كرون من الفئران.