Study the Biological, Histopathological and Immunological Changes of Male Albino Rats Inflicted with Acute Ulcerative Colitis Disease Whole Barley Flour Effect

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ABSTRACT

Polysaccharides are one of the most potent Hull-less barley substances exhibiting anti-inflammatory and immunomodulatory. The aim of present study were to determine whether orally feeding hull-less barley *Hordeum vulgare* could attenuate or prevent the development of experimental colitis in rats. Colonic inflammation was induced in rats by treatment with 5% dextran sulfate sodium (DSS) for 7 days. Rats were fed on the basal diet by replacing (5%, 10%, 15% & 20%) proportions from corn starch (in the composition of basal diet) by the same proportions of whole barley flour (WBF) for 28 days. Colonic damage macroscopically and histopathologically evaluated. Inflammation was assessed by changes in colon length, TNF-α levels released by colonic samples in organ culture and myeloperoxidase (MPO) activity. Levels of pro-inflammatory (IL-1β), (INF-γ) and anti-inflammatory (IL-10) cytokines in colonic samples were determined by enzyme-linked immunosorbent assay (ELISA). Inhibitor subunit of nuclear factor-kappa B (IkBα) in a cell cytoplasm of colonic samples was determined by (ELISA). Hull-less barley attenuated and prevented the development of symptoms associated with DSS-induced colitis. Low proportions of whole barley flour as a product of hull-less barley milling blocked colon shortening, suppressed MPO activity and improved macroscopic score in low proportions treatment groups (especially 5%WBF). Alike, whole barley flour attenuated erythrocytes and leukocytes profile in complete blood count in rat blood serum. In addition, histopathological damage from colitis was reduced by low proportions of (WBF). The tissue levels of TNF-α, IL-1β and IL-10 proteins were significantly decreased and correlated with degrees of inflammation and macroscopic score in low and moderate groups proportions.

**Key words:** Whole barley flour, Dextran Sulfate Sodium (DSS), Colitis, Immunomodulatory.
INTRODUCTION

Both UC and CD when present in the colon, generate a similar symptom profile which can include diarrhea, rectal bleeding, abdominal pain and weight loss (Sands, 2004). Although the pathogenesis of IBD remains unknown, it is described a multifactorial disease that involves genetic and environmental components (Danese and Fiocchi, 2006).

Inflammatory bowel disease (IBD) including Crohn’s Disease (CD) and Ulcerative Colitis (UC) are chronic inflammatory disorders of the gastrointestinal tract thought to result from inappropriate inflammatory responses to intestinal microbes (Abraham and Cho, 2009). The pathogenesis of intestinal bowel disease IBD involves multiple cell types of the mucosal immune system, including intestinal epithelial cells, innate immune cells such as macrophages, neutrophils, T cells and innate lymphoid cells (Wallace et al., 2014).

Naked or hull-less barley (Hordeum vulgare) is a good source of dietary fiber providing soluble and insoluble dietary fiber fractions (Bhatty, 1999; Izydorczyk et al., 2000). Mixed linkage (1→3), (1→4)\-β-D-glucan are a major part of the soluble dietary fiber (SDF) in barley. A previous study showed that the total β-glucan content is higher, whereas the insoluble dietary fiber content is significantly lower in naked barley (Xue et al., 1997) compared to hulled barley genotypes. High-amylase and waxy hulless barley contains approximately 7 or 8% β-glucans, whereas regular hulless barley comprise significantly 4.6% (Gao et al., 2009; Tiwari and Cummins, 2008).

B-glucans are potent immunomodulator with effect on both innate and adaptive immunity. The ability of the innate immune system is on cell response to quickly recognize an response to an invading pathogens for controlling infection. Dectine-1 is expressed on cells responsible for the innate immune response and has been found to be macrophages, neutrophils and dendritic cells (Taylor et al., 2002). The measurement of myeloperoxidase (MPO) activity is considered a quantitative and sensitive assay for assessing acute intestinal inflammation. MPO is a mammalian pro-oxidant enzyme mainly released by activated neutrophils, which catalyzes the conversion of hydrogen peroxide (H₂O₂) and chloride to hypochlorous acid (HOCl) which in turn readily oxidizes key molecules, such as proteins and lipids (Brennan et al., 2003; Wiersema et al., 2008). Barley or yeast β-glucan also showed an immunostimulatory effect mediated by the activation of neutrophils, macrophages, monocytes and natural killer cells through specific receptors CR3 CD11b/CD18 (Vetvicka et al., 1996) and through β-glucan receptor. This was associated with stimulated production of cytokines, such as TNF-α and IL-1, resulting increased immunological surveillance (Thorton et al., 1996). Aim of the present study was to investigate the anti-inflammatory effect of different whole barley flour proportions in dextran sulfate sodium (DSS)- induced colitis in rats.
MATERIALS
1. Pathogen-free adult male albino wistar rats, weighing 250-300 g were obtained from Agriculture Research Center (ARC), Giza, Egypt.
2. Dextran sulfate sodium salt, Molecular Weight 40 K Da, Segma Aldrich, Germany.
3. Rat ELISA kits, MPO, TNF-α, IL-1β, IL-10, INF-γ &IkBα from NOVA, Bionoevan Co., China.
4. HTAB, KH₂PO₄, K₂HPO₄, OXFORD LAB.CHEM., India.
5. Paraformaldehyde, MW 30,03, India.
6. Hull-less barley (Hordeum vulgare) obtained from ARC, Giza, Egypt.
7. Casein, vitamins, minerals, cellulose, sucrose, choline chloride & methionine were purchased from Middle East Co., Cairo, Egypt.
8. Corn oil and corn starch were purchased from Menoufia local market.
9. Hemoccult II Dispensapak Plus, coloview system for determination occult blood in stool.

All basal diets during the study were purified, based on AIN-93 M diet (American Society for Nutrition, USA) for maintenance of adult rats, prepared and applied Experimental Animal Housing, ARC, Giza, Egypt.

METHODS
Whole barley flour mill of Hull-less barley cereals at 5%, 10%, 15% & 20% levels replaced the same quantities of corn starch in diets.

Basal diet composition of test rats was according to (Reeves et al., 1993). It was consisted of 15% casein, 10% sucrose, 10% corn oil, 0.2% choline chloride, 0.3% methionine, 1% vitamin mixture, 4% salt mixture and 5% fiber (Cellulose). The reminder was corn starch, the composition of vitamin and mineral mixture was according to Hegested et al., (1941) and Campbell, (1993) respectively.

Experimental design
Forty two adult male albino wistar rats (250-300g) were purchased from ARC, Giza, Egypt. Animals were housing in plastic cages in a light and temperature-controlled room on a 12 to 12 h light-dark cycle, in which the temperature (25°C) and relative humidity (65-70%) were kept constant. The experimental groups were fed the same diet as the control and access to water was allowed ad libitum. After two weeks of acclimatization, animals will divided into six groups with seven animal in each group as follows: Group (1) control negative (-ve), group (2) kept without any treatment as a positive control (+ve), Dextran sulfate sodium (DSS)-induced ulcerative colitis rats [received orally 5% (w/v) dextran sulfate sodium in drinking water] for a period of 7 successive days (Cheah et al., 2013), the remaining groups received orally 5% (w/v) dextran sulfate sodium for a period 7 successive days.
in addition to treatments: Group (3), fed on the basal diet by replacing 5% from corn starch (in the composition of basal diet) by 5% whole barley flour. Consistency, group (4), group (5), group (6) fed on the basal diet by replacing 10%, 15%, 20% from corn starch (in the composition of basal diet) by whole barley flour (WBF) respectively.

1. Disease Activity Index (DAI)

During the duration of the experiment a disease activity index (DAI) scores measured to evaluate the clinical progression of colitis. The DAI is the combined score of weight loss compared to initial weight, stool consistency & bleeding.

Scores are defined as follows:

1.1. Body weight loss

During the experimental period (28) days body weight measured and calculated the percentage (%) of weight as compared to baseline weight using the following formula:

\[(\%) \text{ of body weight loss (BWL)} = \frac{(\text{weight day } X - \text{baseline weight})}{\text{baseline weight}} \times 100\]

(Jérôme et al., 2016).

Body Weight Loss Scores (0-4), o no loss, 1 (1-5%), 2 (5-10%), 3 (10-20%), 4 (> 20%) (Axelsson et al., 1996 & Egger et al., 2000).

1.2. Stool consistency

Each rat was placed in an individual empty cage to collect and score feces for consistency and blood (Jérôme et al., 2016). The colonic damage was quantified by assessing stool consistency and rectal bleeding was previously described by Loher et al., (2014), scores (0-4), 0 normal mean well formed stool, 2 pasty stools, 4 liquid stools that stuck to the anus.

1.3. Faecal occult blood

Faecal occult blood was determined on a Hemoccult II Dispensapak plus Coloview system by two investigators who were blinded to the treatment groups (Benoit et al., 2018).

Faecal occult blood Scores (0-4), 0 no blood in haemoccult, 2 a positive haemoccult, 4 gross bleeding. The sum of these individual scores gave a final mark ranging from 0 for good clinical symptoms to 8 for very ill or severe colitis (Loher et al., 2004)

2. Histopathological assessment
After sacrifice, the whole colon was removed and length was measured. The distal third of the colon was fixed in 4% paraformaldehyde solution. Histological damage scoring was calculated on paraffin-embbedded haematoxylin & eosin stained sections according to Erben et al., (2014).

Blood sample were collected after 12 h fasting at the end of the experiment using the orbital vein and serum separation to separate serum, left to clot at room temperature, then centrifuged for 10 min at 3000 rpm to separate the serum, aspirated and stored frozen at -20°C for analysis (Schermr, 1967). All serum sample were analyzed for determination complete blood count (CBC). The internal organs, colon, spleen, liver, heart & lungs were removed, washed in saline solution and weighted.

3. Separation & cutting of colon histopathological and immunological assays (Dieleman et al., 1994; Jérôme et al., 2016)

The colon removed by cutting just after the ileocecal junction and the terminal end of the rectum. A representatives pictures of colons took and colon length measured. The colon were cut to three parts (proximal, middle & distal), proximal parts for analyzing cytokines levels, TNF-α, IL-1β, IL-10, INF-γ & IκBα. The middle parts for analyzing Myeloperoxidase (MPO) enzyme. The distal parts for assessing histopathology, colon fragments were cut about 1 cm.

Dextran sulfate sodium (DSS) model is not dependent on adaptive immunity this useful to the analyze the contribution of the of the innate immune system (Dieleman et al., 1994).

4. Myeloperoxidase(MPO) enzyme assay in rat colonic tissues (Manufacturer’s Protocol, NOVA, Bioneovan Co., Ltd, CHINA)

ELISA kits used ELISA sandwich as the method for determining Myeloperoxidase (MPO) levels in rat colonic tissues. Tissues sample are cut, weighed, (300mg) frozen in liquid nitrogen and directly stored at -80°C for future use. The tissue samples were homogenized after adding Phosphate Buffer Saline PBS (PH 7.4). Samples were operated at 4°C. The supernatant collected carefully after centrifuging for 20 min at 2000-3000 rpm. The supernatant aliquoted and used for ELISA assay.

5. Determination of cytokines in rat colonic tissue samples

Rat Tumor necrosis factor α (TNF-α), Rat Interleukin-1β (IL-1β), Rat Interleukin 10(IL-10), Rat Interferon-gamma (IFN-γ), Rat Inhibitory Subunit Of NF Kappa B Alpha (IκBα), ELISA Kits assessed according to (Manufacturer’s Protocol, NOVA, Bioneovan Co., Ltd, China) ELISA kits used ELISA sandwich as the method for determining (TNF-α), (IL-1β), (IL-10), (IFN-γ) & (IκBα) levels in rat colonic tissues. Tissues sample are cut, weighed, (300mg) frozen in liquid nitrogen and directly stored at -80°C for future use. The tissue samples were homogenized after adding PBS (PH 7.4).
Samples were operated at 4°C. The supernatant collected carefully after centrifuging for 20 min at 2000-3000 rpm. The supernatant aliquoted and used for ELISA assay (Manufacturer’s Protocol, NOVA, Bioneovan Co., Ltd, China).

RESULTS
1. Effects of different whole barley flour (WBF) proportions on Disease Activity Index (DAI) in colitis rats
1.1. Body Weight Loss
   Body weight loss was calculated as the percent differences between the initial body weight (day 1) and (day 28). Table (1) & Fig. (1) show the effects of different whole barley flour proportions on body weight loss of colitis rats. It could be observed that the mean BWL score of control (+) group was higher than colitis rats treated with WBF groups \(1.86\pm0.007\) & \((1.26\pm0.001, 1.40\pm0.004, 1.56\pm0.006, 1.62\pm0.005)\)g respectively which revealed significant differences with percent decrease -32.26\%, -24.73\%, -16.13\%, -12.90\% of treated groups (3, 4, 5, 6) as compared to control (+) group.

   The highest percent score of decrease was group 3 (5% whole barley flour) while, the lowest percent score of decrease was group 6 (20% whole barley flour) when compared control (+).

1.2. Stool Consistency
   Table (1) & Fig. (1) show the effect of different whole barley flour proportions on stool consistency in colitis rats. It could be noticed that the mean stool consistency score of control (+) group was higher than colitis treated with WBF groups \(2.45\pm0.006\) & \((1.82\pm0.003, 1.90\pm0.004, 1.94\pm0.007, 1.98\pm0.001)\)respectively which revealed significant differences with percent decrease -25.71\%, -22.45\%, -20.82\%, -19.18\% of treated groups (3, 4, 5, 6) as compared to control (+) group. The highest percent score of decrease was group 3 (5% whole barley flour) and the lowest percent score decrease was group 6 (20% whole barley flour).

1.3. Occult Blood
   Table (1) & Fig. (1) illustrate the effects of different whole barley flour (WBF) proportions on occult blood in colitis rats. It could be shown that the mean score of control (+) group was the highest being \(1.60\pm0.008\) while was less for colitis rat groups treated with WBF being \((1.09\pm0.009, 1.18\pm0.008, 1.24\pm0.004, 1.35\pm0.003)\)g respectively which revealed significant differences with percent decrease-31.88\%, -26.25\%, -22.50\%, -15.63\% of treated groups (3, 4, 5, 6) as compared to control (+) group. The highest percent score of decrease was group 3 (5% whole barley flour) and the lowest percent score
decrease was group 6 (20% whole barley flour) as compared to control (+) group.

Table (1): Effects of different whole barley flour (WBF) proportions on DAI in treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Body Weight Loss (Score) Mean ±SD</th>
<th>Stool Consistency (Score) Mean ±SD</th>
<th>Occult Blood (Score) Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Group 1:</td>
<td>Control –ve</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% Change of Positive control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 2:</td>
<td>Control +ve</td>
<td>1.86±0.007</td>
<td>2.45±0.006</td>
<td>1.60±0.008</td>
</tr>
<tr>
<td>% Change of Positive control</td>
<td>1.09±0.009</td>
<td>1.82±0.003</td>
<td>1.09±0.009</td>
<td></td>
</tr>
<tr>
<td>Group 3:</td>
<td>WBF (5%)</td>
<td>1.26±0.001</td>
<td>1.82±0.003</td>
<td>1.09±0.009</td>
</tr>
<tr>
<td>% Change of Positive control</td>
<td>32.26±0.001</td>
<td>25.71±0.006</td>
<td>31.88±0.006</td>
<td></td>
</tr>
<tr>
<td>Group 4:</td>
<td>WBF (10%)</td>
<td>1.40±0.004</td>
<td>1.90±0.004</td>
<td>1.18±0.008</td>
</tr>
<tr>
<td>% Change of Positive control</td>
<td>24.73±0.004</td>
<td>22.45±0.004</td>
<td>26.25±0.004</td>
<td></td>
</tr>
<tr>
<td>Group 5:</td>
<td>WBF (15%)</td>
<td>1.56±0.006</td>
<td>1.94±0.007</td>
<td>1.24±0.004</td>
</tr>
<tr>
<td>% Change of Positive control</td>
<td>16.13±0.004</td>
<td>20.82±0.007</td>
<td>22.5±0.008</td>
<td></td>
</tr>
<tr>
<td>Group 6:</td>
<td>WBF (20%)</td>
<td>1.62±0.005</td>
<td>1.98±0.001</td>
<td>1.35±0.003</td>
</tr>
<tr>
<td>% Change of Positive control</td>
<td>12.90±0.005</td>
<td>19.18±0.001</td>
<td>15.63±0.003</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>0.008</td>
<td>0.008</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

Values in each column with different letters are significantly different (P<0.05).
**Figure (1):** Effects of different whole barley flour (WBF) proportions on disease activity index (DAI) in treated rats

### 2. Effect of different whole barley flour (WBF) proportions on the length of colon in male albino wistar rats

Table (2), figure (2) and Photo (1) show the effect of different Whole barley flour (WBF) proportions on the length of colon in treated rats. It could be shown that the mean value of control (+) group was lower than control (−) group being 12.6±0.09 & 14.8±0.01 cm respectively which revealed significant differences with percent increase 17.46% of control (−) group as compared to control (+). Other groups indicated significant increases as compared control (+) group. The mean values were 15.5±0.05 & 13.9±0.08 & 13.7±0.04 & 12.58±0.003 cm for 5% WBF, 10% WBF, 15% WBF, 20% WBF respectively. The percent of increases were 23.02%, 10.32%, 8.73% for groups 3, 4, 5 respectively. Groups 2 & 6 show nonsignificant differences between them. The best length of colon recorded for group 5 (5% WBF) for colitis rats when compared to control (+).

**Figure (1):** Effects of different whole barley flour (WBF) proportions on colon length in treated rats

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>Length of colon (cm)</th>
<th>%Change of Control (+ve)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td><strong>Group1:</strong> Control –ve</td>
<td>14.8±0.01</td>
<td>17.46</td>
</tr>
<tr>
<td><strong>Group2:</strong> Control +ve</td>
<td>12.6±0.09</td>
<td>0</td>
</tr>
<tr>
<td><strong>Group3:</strong> Whole barley flour (5%)</td>
<td>15.5±0.05</td>
<td>23.02</td>
</tr>
<tr>
<td><strong>Group4:</strong> Whole barley flour (10%)</td>
<td>13.9±0.08</td>
<td>10.32</td>
</tr>
<tr>
<td><strong>Group5:</strong> Whole barley flour (15%)</td>
<td>13.7±0.04</td>
<td>8.73</td>
</tr>
<tr>
<td><strong>Group6:</strong> Whole barley flour (20%)</td>
<td>12.58±0.003</td>
<td>-0.16</td>
</tr>
<tr>
<td><strong>LSD</strong></td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

Values in each column with different letters are significantly different (P<0.05).

Dysfunction of intestinal barrier lead to increased intestinal permeability that have been associated with the pathogenesis of IBD inflammatory bowel disease (Kiesler et al., 2015)
During DSS administration, mice can exhibit pronounced weight loss (about 5% to 10% reduction by day 5), altered stool consistency leading to diarrhea and hematochezia. A significant physiological indicator of animal stress and imminent demise occurs if animal weight loss is greater than 20% of the original weight (Kim et al., 2012).

Murine colitis result from administration of 40–50 Kda DSS added in drinking water. In the DSS model, the sulfated polysaccharides do not directly induce intestinal inflammation, but rather acts a direction chemical toxin to colonic epithelium resulting in intestinal epithelial injury (Okayasu et al., 1990; Poritz et al., 2007; Wirtz et al., 2007; Perše et al., 2012; Samak et al., 2015).

β-glucans are found in food including cereals, mushrooms and yeast, as well as in some bacteria (Diller and Mankowski, 1963). β-glucans are complex glucose polymers containing a backbone of β-1,3-linked and β-1,4-D-glucose molecules (Mueller et al., 2000).

![Colon length (cm)](image)

**Figure (2):** Effect of different whole barely flour (WBF) proportions on the length of colon parameters in Colitis wistar rats

Dectin-1 is constitutively activated by the ligands in the intestine and suppresses Treg differentiation. Thus, inhibition of Dectin-1 may be fundamentally beneficial for the host under healthy conditions; it suppresses inflammation caused by irritants, allergens, or infections by increasing Treg cells. However, at the same time, excessive Dectin-1 inhibition may cause the host to be susceptible to fungal infection in the intestine, resulting in the aggravation of colitis. Excessive Dectin-1 activation through β-glucans in foods or fungi may also aggravate colitis by suppressing development of Treg cells even in fungus-infected individuals (Ce Tang et al., 2015).
Photo 2: Effects of various whole barley flour proportions on length of colon in different groups.
The uptake of orally administered β-glucans, and biologic effect thereof, has been highly controversial. Although it is now widely accepted that orally administered β-glucans may enhance host immunity, it is still not established whether β-glucans may act directly on the gastrointestinal mucosa or if entry the blood stream is feasible and required to mediate biological effects. Gastrointestinal absorption of orally administered β-glucans has been addressed in a very limited number of publications. Hong et al. (2004) reported uptake of particulate β-glucan from the gut mediated by intestine macrophages that

internalized the β-glucan particle, circulated throughout the body, and subsequently released bioactive soluble β-glucan into circulation (Hong et al., 2004).

In other study the hypothesis that systemic β-glucan treatment would result in enhanced migration of neutrophils into a site of inflammation and improve antimicrobial function was tested in a model of acute inflammation in rats. Animals treated with β-glucan showed a 66±6% and 186±42% increase in wound cell number recovered 6 and 18 h post wounding respectively. Increased migration however, did not correlate with increased chemo attractant content of wound fluid, alterations in neutrophils –induced loss of endothelial barrier function, or changes in neutrophils adhesion to endothelial cells. Studies also showed a priming effect for chemotaxis and respiratory burst in circulation neutrophils isolated from β-glucan-treated animal (Leblanc et al., 2006).

Low dosages of whole barley flour prevented colon shortening. Colon shortening is always found in ulcerative colitis patients and can be a serve as an indirect marker of colonic inflammation (Chung et al., 2007). A dosage of whole barley flour 5% was the best result, therefore in 2006 the Food and Drug Administration (FDA) approved a health claim on the positive effect of β-glucans from barley on cholesterol reduction and risk of heart disease in daily consumption of 3g of soluble β-glucans (EFSA, 2009; EFSA, 2011).

On the one hand, the claim to the effect of β-glucans on maintaining a normal cholesterol level that can be used for foods containing at least 1 g of β-glucans in one quantified portion and beneficial effect is achieved with a daily intake of 3 g β-glucans. The second claim concerns the beneficial effects of β-glucans oats and barley on blood glucose (EFSA, 2009; EFSA, 2011).

3. Effect of different whole barely flour (WBF) proportions on organs weight in male albino wistar rats.
3.1. Colon Weight
Table (3) & Fig. (3) show the effect of WBF proportions on colon weight (g) colitis rats. It could be noticed that the mean value control (+) group was higher than control (−) group being 5.1±0.01 & 4.80±0.05 g respectively which revealed significant differences with percent of decrease -5.88% of control (−) group as compared to control (+) group.

All other groups showed significant differences as compared to control (+) group. The value were 4.70±0.07 & 4.89±0.002 & 4.90±0.09 & 4.96±0.006 g for 5% WBF, 10% WBF, 15% WBF, 20% WBF respectively. The percent of decrease were -7.84%, -4.12%, -3.92%, -2.75% respectively. Groups 4, 5 showed no significant differences between them. Group 3 (5%WBF) recorded the best group for colon weight of colitis rats. These result agree with that of Antonio et al., (2000); Andrea et al., (2018). Colitic mice had a significant increase in colon weight, as well as an increase in the ratio of colon weight to length. In present work the ratio of colon weight to colon length were 32.4% and 40.5% for control (-) and control (+) respectively. These ratios revealed significant difference between them.

3.2. Spleen Weight (g)

Table (3) & Fig. (3) depict the effect of different whole barley flour (WBF) proportions on spleen weight (g) in colitis rats. It could be noticed that the mean value of control (+) group was higher than control (−) group being 1.01±0.08 & 0.89±0.002 g respectively which revealed decreased differences with percent of decrease -11.88% of control (−) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The value were 0.76±0.005, 0.80±0.009, 0.90±0.002, 0.93g for 5% WBF, 10% WBF, 15% WBF, 20% WBF respectively.

The percent of decrease were -24.75%, -20.79%, -10.89%, -7.92% respectively. Groups 1, 5, 6 showed no significant differences among them. Groups 3 &4 showed no significant differences between them. Group 3 (5% WBF) recorded the best group for spleen weight of colitis rats showing lower value than when compared to control (−) group. These results were in agreement with that of Antonio et al., (2000); Andrea et al, (2018) who was recorded significant differences between normal and DSS-induced colitis in which spleen weight in DSS-induced colitis was higher than normal. In present work (Table 3).

3.3. Liver Weight (g)

Table (3) & Fig. (3) show the effect of different whole barley flour(WBF) proportions on liver weight (g) in colitis rats. It could be observed that the mean value of control (+) group was significantly higher than control (−) group being 8.59±0.007 & 6.99±0.005g respectively which revealed significant
difference with percent of decrease -18.63% of control (-) group as compared to control (+).

All other groups showed significant differences as compared to control (+) group. The values were 7.55±0.009, 7.77±0.008, 7.80±0.004, 8.1±0.84g for 5% WBF, 10% WBF, 15% WBF, 20% WBF respectively. The percent of decrease -12.69%, -9.55%, -9.20%, -5.70% for above mentioned groups respectively. Group 3 (5% WBF) recorded the best group for liver weight of colitis rats.

3.4. Heart Weight (g)

Table (3) & Fig. (3) show the effect of different whole barley flour (WBF) proportions on heart weight (g) in colitis rats. It could be depicted that the mean value of control (+) group was higher than control (-) group being 0.87±0.008 & 0.79±0.004g respectively which revealed significant difference with percent decrease -9.20% of control (-) group as compared control (+) group.

All other groups showed significant differences with control (+) group. The value were 0.78±0.002, 0.79±0.009, 0.82±0.007, 0.84±0.005g for 5% WBF, 10% WBF, 15%WBF, 20% WBF respectively. Groups 1, 3, 4 showed no significant differences among them. Group 3 (5% WBF) recorded the best group for heart weight of colitis rats.

3.5. Lungs Weight (g)

Table (3) & Fig. (3) show the effect of different whole barley flour (WBF) proportions on lungs weight (g) in colitis rats. It could be noticed that the mean value of control (+) group was higher than control (-) group being 1.27±0.007 &1.19±0.001g respectively which revealed significant difference with percent decrease -6.30% of control (-) as compared control (+) group. All other groups showed significant differences with control (+) group. The value were 1.16±0.006, 1.18±0.003, 1.21±0.005, 1.22±0.009g for 5% WBF, 10% WBF, 15% WBF, 20% WBF respectively. Groups 5 &6 showed no significant difference between them. Group 3 (5% WBF) recorded the best group for lungs weight of colitis rats.

It could be noticed that whole barley flour (5%) ameliorated the internal organs weights rather other groups. Complement Receptor (CR3) is highly expressed on Neutrophils, Monocytes and Nature Killer cells and less present macrophages (Zimmerman et al., 1998). Barley β-glucan is primarily linear with large regions of β-(1→4) glucan linkages separates shorter stretches of β-(1→3) structures. B-(1→3), (1→4) glucan from barley has shown that they function through stimulation of granulocytes (neutrophils and eosinophils), monocytes, macrophages and NK cells (Ross, 2000).

The animals in groups treated with DSS showed increases in liver and cecum weight (Vrublova et al., 2010).
Table (3): Effect of different whole barely flour (WBF) proportions on organs weight (g) in treated male albino wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Colon</th>
<th>Spleen</th>
<th>Liver</th>
<th>Heart</th>
<th>Lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Group1</td>
<td>Control (−)</td>
<td>4.80° ±0.05</td>
<td>0.89° ±0.002</td>
<td>6.99° ±0.005</td>
<td>0.79° ±0.004</td>
<td>1.19° ±0.001</td>
</tr>
<tr>
<td>%Change of Positive Control</td>
<td>-5.88</td>
<td>-11.88</td>
<td>-18.63</td>
<td>-9.20</td>
<td>-6.30</td>
<td></td>
</tr>
<tr>
<td>Group2</td>
<td>Control (+)</td>
<td>5.1ª ±0.01</td>
<td>1.01ª ±0.08</td>
<td>8.59ª ±0.007</td>
<td>0.87ª ±0.008</td>
<td>1.27ª ±0.007</td>
</tr>
<tr>
<td>%change of Positive control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Group3</td>
<td>WBF(5%)</td>
<td>4.70ª ±0.07</td>
<td>0.76ª ±0.005</td>
<td>7.55ª ±0.008</td>
<td>0.78ª ±0.002</td>
<td>1.16ª ±0.006</td>
</tr>
<tr>
<td>%Change of Positive Control</td>
<td>-7.84</td>
<td>-24.75</td>
<td>-12.69</td>
<td>-10.34</td>
<td>-8.66</td>
<td></td>
</tr>
<tr>
<td>Group4</td>
<td>WBF(10%)</td>
<td>4.89ª ±0.002</td>
<td>0.80ª ±0.009</td>
<td>7.77ª ±0.008</td>
<td>0.79ª ±0.009</td>
<td>1.18ª ±0.003</td>
</tr>
<tr>
<td>%change of Positive control</td>
<td>-4.12</td>
<td>-20.79</td>
<td>-9.55</td>
<td>-9.20</td>
<td>-7.09</td>
<td></td>
</tr>
<tr>
<td>Group5</td>
<td>WBF(15%)</td>
<td>4.90ª ±0.09</td>
<td>0.90ª ±0.002</td>
<td>7.82ª ±0.004</td>
<td>0.82ª ±0.007</td>
<td>1.21ª ±0.005</td>
</tr>
<tr>
<td>%Change of Positive Control</td>
<td>-3.92</td>
<td>-10.89</td>
<td>-9.20</td>
<td>-5.75</td>
<td>-4.72</td>
<td></td>
</tr>
<tr>
<td>Group6</td>
<td>WBF(20%)</td>
<td>4.96ª ±0.006</td>
<td>0.93ª ±0.006</td>
<td>8.08ª ±0.006</td>
<td>0.84ª ±0.005</td>
<td>1.22ª ±0.009</td>
</tr>
<tr>
<td>%Change of Positive Control</td>
<td>-2.75</td>
<td>-7.92</td>
<td>-5.70</td>
<td>-3.45</td>
<td>-3.94</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>0.09</td>
<td>0.06</td>
<td>0.61</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values in each column with different letters are significantly different (P<0.05).
4.1 Effect of different whole barely flour (WBF) proportions on Immune cells in Colitis rats.

4.1.1. Neutrophils

Table (4) & Fig. (4) show the effect of different whole barely flour proportions on Neutrophils cells in Colitis treated rats. It could be noticed that the mean value of control (+) group was higher than control (‒) group, being 21.2±0.1 & 16.8±0.06, cells respectively which illustrated significant differences with percent of decrease -20.75% of control (‒) group as compared to control (+) group.

Groups of colitis treated with 5% WBF, 10%WBF, 15%WBF, 20%WBF revealed significant difference as compared control (+). The mean values were 13.3±0.05, 13.7±0.04, 15.33±0.07 & 19.2±0.01 (1000/μl) respectively. The percent of decrease -37.26%, -35.38%, -27.83% & -7.08% for above mentioned groups. Group 3 (5%WBF) recorded the best group for treatment colitis as compared to colitis treated groups.

4.1.2. Lymphocytes (Segmented neutrophils.)

Table (4) & Fig. (4) show the effect of different whole barely flour proportions on Lymphocytes cells in Colitis treated rats. It could be noticed that the mean value of control (+) group was lower than control (‒) group, being 67.4±0.06 & 72.1±0.07 (1000/μl) respectively which illustrated significant differences with percent of increase 9.50%, 7.86% & 3.86% respectively as compared to control (+) group.
Group 6 (20%WBF) depicted a significant difference with percent of decrease -0.59% of group 6 (20%WBF) as compared to control (+) group. Group 3 (5%WBF) recorded the best value for treatment colitis as compared to colitis treated groups.

4.1.3. Monocytes
Table (4) & Fig. (4) show the effect of different whole barely flour proportions on Monocytes cells in Colitis treated rats. It could be noticed that the mean value of control (+) group was higher than control (–) group, being 7.8±0.08 & 6.8±0.06 (1000/µl) respectively with significant difference as compared to control (+) group. The percent of decrease -12.82% of control (–) group as compared to control (+) group.

Groups 4, 5, 6 for 10%WBF, 15%WBF, 20%WBF, showed significant differences with percent of increases 8.97%, 8.97% &21.79% respectively for above mentioned as compared to control(+) group. Group 3 (5%WBF) illustrated a significant difference as compared to control (+) group. The mean value being 6.8±0.03(1000/µl), the percent of decrease being -12.82% of group 3 (5%WBF).

Groups 4, 5 for (10%WBF) & (15%WBF) respectively, revealed insignificant differences between them. Consistency, control (–) group& group 3 (5%WBF) showed insignificant differences between them. Group 3 (5%WBF) recorded the best group for treatment colitis as compared to colitis treated groups.

4.1.4. Eosinophils
Table (4) & Fig. (4) show the effect of different whole barely flour proportions on Eosinophils cells in Colitis treated rats. It could be noticed that the mean value of control (+) group was higher than control (–) group, being 1.6±0.08 & 1.3 ±0.01(cells/mcl) respectively, which revealed a significant difference with percent of decrease was -18.75% of control (-) group as compared to control (+) group. Groups 3, 4 & 5 for 5%WBF, 10% WBF & 15% WBF showed nonsignificant differences among them.

Group 3 (5%WBF) recorded the highest group. The percent of decrease being -37.5%, as compared to control (+) group. Group 3 (5%WBF) recorded the best group for treatment colitis as compared to colitis treated groups.

Barley-derived (1→3), (1→4)-β-D-glucan have undergone clinical testing primarily for their cholesterol-reducing activity 22 (Nicolosi et al., 1999).

Glucan-binding receptors in human leucocytes include lactosylceramide23, dectin-1 (Brown et al., 2002), and the complement receptor 3 (CR3) (Thornton et al., 1996). Most recent studies indicate that complement receptor3 (CR3) is the major receptor mediating the immunological effects of β-glucans (van Bruggen et al., 2009; Huang et al., 2012).
Based on mostly animal data, β-glucans enter the proximal small intestine rapidly and are captured by the macrophages after oral administration. The β-glucans are then internalized and fragmented into smaller sized β-glucans and are carried to the marrow and endothelial reticular system. The small β-glucans fragments are then released by the macrophages and taken up by the circulating granulocytes, monocytes, dendritic cells (Rice et al., 2005).

Table (4): Effect of different whole barely flour (WBF) proportions on Immune cells in Colitis rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>SEG Mean ±SD</th>
<th>LYM Mean ±SD</th>
<th>MON Mean ±SD</th>
<th>ESO Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group1 Control (−)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Change of Control (+)</td>
<td>-20.75 ±0.06</td>
<td>-72.1 ±0.07</td>
<td>6.8 ±0.06</td>
<td>1.3 ±0.01</td>
<td></td>
</tr>
<tr>
<td><strong>Group2 Control (+)</strong></td>
<td>21.2 ±0.01</td>
<td>67.4 ±0.06</td>
<td>7.8 ±0.08</td>
<td>1.6 ±0.08</td>
<td></td>
</tr>
<tr>
<td>% change of Control (+)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Group3 Whole barley flour (5%)</strong></td>
<td>13.3 ±0.05</td>
<td>73.8 ±0.04</td>
<td>6.8 ±0.03</td>
<td>1 ±0.09</td>
<td></td>
</tr>
<tr>
<td>% Change of Positive control</td>
<td>-37.26 ±0.05</td>
<td>9.50 ±0.04</td>
<td>-12.82 ±0.05</td>
<td>-37.5 ±0.07</td>
<td></td>
</tr>
<tr>
<td><strong>Group4 Whole barley flour (10%)</strong></td>
<td>13.7 ±0.04</td>
<td>72.7 ±0.02</td>
<td>8.5 ±0.04</td>
<td>1.5 ±0.07</td>
<td></td>
</tr>
<tr>
<td>% Change of Positive control</td>
<td>-35.38 ±0.02</td>
<td>7.86 ±0.04</td>
<td>8.97 ±0.07</td>
<td>-6.25 ±0.04</td>
<td></td>
</tr>
<tr>
<td><strong>Group5 Whole barley flour (15%)</strong></td>
<td>15.33 ±0.07</td>
<td>70 ±0.05</td>
<td>8.5 ±0.01</td>
<td>1.5 ±0.04</td>
<td></td>
</tr>
<tr>
<td>% Change of Positive control</td>
<td>-27.83 ±0.08</td>
<td>3.86 ±0.04</td>
<td>8.97 ±0.04</td>
<td>-6.25 ±0.04</td>
<td></td>
</tr>
<tr>
<td><strong>Group6 Whole barley flour (20%)</strong></td>
<td>19.2 ±0.01</td>
<td>67 ±0.08</td>
<td>9.5 ±0.05</td>
<td>1.5 ±0.06</td>
<td></td>
</tr>
<tr>
<td>% Change of Positive control</td>
<td>-7.08 ±0.05</td>
<td>-0.59 ±0.08</td>
<td>21.79 ±0.05</td>
<td>-6.25 ±0.06</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

Values in each column with different letters are significantly different (P<0.05)
4.2. Effect of different whole barley flour proportions on WBCs and PLTs levels in Colitis wistar rats.

4.2.1. White Blood Cells (WBCs)

Table (5) & Fig. (5) show the effect of different Whole Barley Flour proportions on WBCs levels in Colitis wistar rats. It could be noticed that the mean value of control (+) group was higher than control (−) group, being 8.64±0.004 & 7.4±0.09 respectively which illustrated significant differences with percent decrease -14.3% of control (−) group as compared control (+). All other groups showed significant differences as compared to control (+) group. The values were 6.6±0.02, 7.37±0.08, 7.52±0.08, 8.68±0.003 respectively for 5% WBF, 10% WBF, 15% WBF, 20% WBF respectively. The percent of decrease -23.61%, -14.70%, -12.96%, 0.46% respectively. Group 3(5% WBF) recorded the best group for WBCs of colitis rats even when compared to control (+) group.

4.2.2 Platelets

Table (5) & Fig. (6) show the effect of different Whole Barley Flour proportions on PLTs levels in Colitis wistar rats. It could be noticed that the mean value of control (+) group was higher than control (−) group, being 1059.4±7.42 & 943.7±5.15 thousands/cmm respectively which illustrated significant differences with percent decrease -10.92 % of control (−) group as compared control (+) group.

All other groups revealed significant differences as compared to control (+). The values were 929.5±2.89, 935.7±3.26946.7±2.64, 272.5±3.58...
thousands/mm for 5% WBF, 10% WBF, 15% WBF, 20% WBF respectively. The percent of decrease were -12.26%, -11.68%, --10.64%,-8.20% thousands/mm respectively for the above mentioned groups. Best groups seem to be recorded for group3 (WBF 5%).

B-(1→3) glucans are able to interact directly with leukocytes and platelets 40,39Higher β-glucans content is not desirable even for barley intended for monogastric feed (piglets, poultry) that cause digestive problems and reduce nutrient utilization (Prugar et al., 2008).

From data of table (5) WBF 5% group was the best Mean

Table (5): Effect of different whole barley flour proportions on WBCs and PLTs levels in Colitis wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>White Blood Cells (WBCs)</th>
<th>Platelet (PLTs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>%Change of Control+ve</td>
</tr>
<tr>
<td>Group1: Control –ve</td>
<td>7.4 ±0.09</td>
<td>-14.35</td>
</tr>
<tr>
<td>Group2: Control +ve</td>
<td>8.64 ±0.004</td>
<td>0</td>
</tr>
<tr>
<td>Group3: Whole barley flour (5%)</td>
<td>6.6 ±0.02</td>
<td>-23.61</td>
</tr>
<tr>
<td>Group4: Whole barley flour(10%)</td>
<td>7.37 ±0.08</td>
<td>-14.70</td>
</tr>
<tr>
<td>Group5: Whole barley flour(15%)</td>
<td>7.52 ±0.003</td>
<td>-12.96</td>
</tr>
<tr>
<td>Group6: Whole barley flour(20%)</td>
<td>8.68 ±0.08</td>
<td>0.46</td>
</tr>
<tr>
<td>LSD</td>
<td>0.096</td>
<td>7.97</td>
</tr>
</tbody>
</table>

Values in each column with different letters are significantly different (P<0.05).
Figure (5): Effect of different whole barley flour (WBF) proportions on white blood cells (WBCs) in treated rats

Figure (6): Effect of different whole barley flour (WBF) proportions on Platelets (PLTs) in treated rats
4.3. Effects of different whole barley flour proportions on red blood cells (Erythrocytes) in treated rats.

4.3.1. Red Blood Cells (RBCs)

Table (6) & Fig. (7) show the effects of different whole barley flour (WBF) proportions on Red Blood Cells (Erythrocytes) in treated rats. It could be noticed that the mean value of control (+) group was lower than control (−) group, being 7.6±0.09 & 8.03±0.005 million/cmm respectively, which illustrated significant differences with percent of increase 5.66% of control (−) group as compared to control (+) group.

Groups 3, 4, 5 revealed significant differences as compared to control (+) group. The values were 7.98±0.006, 7.4±0.04, 7.9±0.02, million/cmm for 5% WBF, 10% WBF, 15% WBF respectively. The percent of increase were 5%, 3.95%, -2.63% for the above mentioned groups. Group 6 revealed significant differences with percent of decrease was -5.26% of group 6 (20% WBF) as compared to control (+). Group 3 (5% WBF) recorded the best group for colitis rats as compared to control (+) group.

4.3.2. Hemoglobin (HGB)

Table (6) & Fig. (7) show the effects of different whole barley flour proportions on Hemoglobin in treated rats. It could be noticed that the mean value of control (+) group was lower than control (−) group, being 13.72±0.002 & 14.4±0.09 g/dl respectively, which control (−) group as compared control (+) group.

Groups 3, 4, 5 revealed significant differences as compared control (+) group. The values were 14.5±0.04, 14.3±0.08, 13.9±0.05 g/dl for 5% WBF, 10% WBF, 15% WBF respectively. The percent of increase were 6.13%, 4.23%, 1.31% respectively for the above mentioned groups. Group 6 revealed significant difference with percent of decrease was -3.79% of group 6 (20% WBF) as compared control (+) group. Group 3 (5% WBF) recorded the best group for colitis rats when compared to control (+) group.

The various factors which causes ID (iron deficiency anemia) in IBD (inflammatory bowel disease) patients are (1) blood loss from the intestinal mucosal ulceration (common in UC) ulcerative colitis, (2) decreased absorption of the iron secondary to surgical resection (common in CD) crohn’s disease, (3) reduced dietary intake and (4) inflammatory cytokines (IL-1, IL-6, TNF-A) mediated hepcidin over expression which causes ferroportin degradation and consequential reduced iron release from the enterocytes to the blood stream (Kulnigg et al., 2006; Stein et al., 2010; Murawska et al., 2016).

4.3.3. Hematocrit (HCT)

Table (6) & Fig. (7) show the effects of different whole barley flour proportions on Hematocrit in treated rats. It could be noticed that the mean value of control (+) group was lower than control (−) group, being 39.02±0.009 & 41.8±0.25 % respectively which illustrated significant difference with percent of increase was 7.09% of control (−) group. Groups 3,
4. 5 for 5% WBF, 10% WBF, 15% WBF respectively revealed significant differences as compared to control (+) group. The values were 41.9±0.02, 41.5 ±0.06, 40.01±0.011 % for 5%WBF, 10% WBF,15% WBF respectively with percent of increase were 7.38%, 6.36%, 2.54% respectively for the above mentioned groups. Group 6 (20% WBF) revealed significant difference with percent of decrease was -4.66% of group 6 (20% WBF) as compared to control (+). Group 3 (5% WBF) recorded the best group when even as compared to control (+) group.

4.3.4. Mean Corpuscular Volume (MCV)

Table (6) & (7) show the effects of different whole barley flour (WBF) proportions on Mean Corpuscular Volume in treated rats. It could be noticed that the mean value of control (+) group lower than control (‒) group, being 51.4±0.04 & 52.2±0.02 fl respectively which illustrated significant difference with percent of increase was 1.56% of control (‒) group as compared to control (+) group.

Groups 3, 4, 5 for 5% WBF, 10% WBF, 15% WBF respectively showed significant differences as compared to control (+) group. The value were 52.5±0.05, 52.3±0.03, 52±0.08 fl for 5% WBF, 10% WBF, 15% WBF respectively. The percent of increase were 3.33%, 1.75%, 1.17% respectively for the above mentioned groups.

Group 6 (20% WBF) revealed significant difference with percent of decrease was -0.14% of group 6 (20% WBF) as compared to control (+) group. Group 3 (5% WBF) recorded the best group when even as compared to control (+) group.

4.3.5. Mean Corpuscular Hemoglobin (MCH)

Table (6) & Fig. (7) show the effects of different whole barley flour (WBF) proportions on Mean Corpuscular Hemoglobin in treated rats. It could be shown that the mean value of control (+) group was higher than control (‒) group, being18.18±0.005 & 17.98±0.001pg decrease was showing -1.10% of control (‒) group. Groups 4, 5, 6 for 10% WBF, 15% WBF, 20% WBF showed significant differences as compared to control (+) group. The values were 18.22±0.008, 18.45±0.002, 18.85±0.006 pg respectively. The percent of increase were 0.22%, 1.49%, 3.69% for above mentioned groups.

Group 3 (5% WBF) showed significant difference with percent decrease was -1.10% of group 3 as compared to control (+) group. Groups 1, 3 revealed no significant differences between them. Group 3 (5% WBF) recorded the best group for MCH in treated rats.

4.3.6. Mean Corpuscular Hemoglobin Concentration (MCHC)

Table (6) & (7) show the effects of different whole barley flour (WBF) proportions on Mean Corpuscular Hemoglobin Concentration in treated rats. It
could be shown that the mean value of control (+) group was higher than control (−) group, being 35.22±0.02 & 34.6±0.03g/dl respectively which illustrated significant difference. The percent of decrease was -1.76% of control (−) group as compared to control (+).

Groups 3, 4, 5 for 5% WBF, 10% WBF, 15% WBF showed significant differences with compared to control (+) group. The mean values were 34.3±0.04, 34.7±0.07, 34.8±0.01g/dl for 5% WBF, 10% WBF, 15% WBF respectively with percent of decrease were -2.61%, -1.48%, -1.19% for above mentioned groups.

Group 6, revealed significant difference with percent of increase was 1.08% of 20% WBF group as compared to control(+) group. Group 3(5% WBF) recorded the best group for MCHC in treated rats even when compared to control (+) group.

Dextran sulfate sodium (DSS) causes erosions with complete loss of surface epithelium because of its direct toxic effect on epithelial cells. It causes deformity in the epithelial integrity, thereby increases the colonic mucosal permeability allowing permeation of large molecules such as DSS with molecular weight upto 50,000 Da (Ni et al., 1996; Dharmani et al., 2011; Dai et al., 2013). In DSS dextran sulfate sodium treated groups a reduction in erythrocytes was associated as a decrease in hemoglobin and hematocrit. The decrease in erythrocytes (hemoglobin, hematocrit) was due to blood loss in stool (Vrublova et al., 2010).

Iron-deficiency anemia was highly prevalent in patients with Crohn's disease (69.6%) and ulcerative colitis (76.7%). Anemia of chronic disease in combination with iron deficiency anemia was present in 3% of the patients with Crohn's disease and in 7% of the patients with ulcerative colitis. There was no association between anemia and disease location. In ulcerative colitis, anemia was associated with the disease activity index (Rodrigo et al., 2014).

Naked barley is a good source of dietary fiber providing soluble and insoluble dietary fiber fraction (Bhatty, 1999; Izydorczyk et al., 2000).

Mixed linkage (1→3), (1→4)-β-D-glucans are a major part of soluble dietary fiber in barley. Higher β-glucans content is not desirable even for barley intended for monogastric feed (piglets, poultry) that cause digestive problems and reduce nutrient utilization (Prugar, 2008).
Table (6): Effects of different whole barley flour proportions on red blood cells (Erythrocytes) in treated rats and related values

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBCs $10^6$/cmm</th>
<th>HGB g/dl</th>
<th>HCT ml</th>
<th>MCV fl</th>
<th>MCH pg</th>
<th>MCHC g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Group1 Control (-ve)</td>
<td>8.03 ±0.05</td>
<td>14.4 ±0.09</td>
<td>41.8 ±0.25</td>
<td>52.2 ±0.02</td>
<td>17.98 ±0.01</td>
<td>34.6 ±0.03</td>
</tr>
<tr>
<td>% Change of Control (+ve)</td>
<td>5.66</td>
<td>4.96</td>
<td>7.09</td>
<td>1.56</td>
<td>-1.10</td>
<td>-1.76</td>
</tr>
<tr>
<td>Group2 Control (+ve)</td>
<td>7.6 ±0.09</td>
<td>13.72 ±0.002</td>
<td>39.02 ±0.009</td>
<td>51.4 ±0.04</td>
<td>18.18 ±0.005</td>
<td>35.22 ±0.002</td>
</tr>
<tr>
<td>% Change of Control (+ve)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group3 WBF (5%)</td>
<td>7.98 ±0.06</td>
<td>14.5 ±0.04</td>
<td>41.9 ±0.02</td>
<td>52.5 ±0.05</td>
<td>17.98 ±0.007</td>
<td>34.3 ±0.04</td>
</tr>
<tr>
<td>% Change of Control (+ve)</td>
<td>5</td>
<td>6.13</td>
<td>7.38</td>
<td>3.33</td>
<td>-1.10</td>
<td>-2.61</td>
</tr>
<tr>
<td>Group4: WBF (10%)</td>
<td>7.9 ±0.02</td>
<td>14.3 ±0.08</td>
<td>41.5 ±0.06</td>
<td>52.3 ±0.03</td>
<td>18.22 ±0.008</td>
<td>34.7 ±0.07</td>
</tr>
<tr>
<td>% Change of Control (+ve)</td>
<td>3.95</td>
<td>4.23</td>
<td>6.36</td>
<td>1.75</td>
<td>0.22</td>
<td>-1.48</td>
</tr>
<tr>
<td>Group5: WBF (15%)</td>
<td>7.4 ±0.04</td>
<td>13.9 ±0.05</td>
<td>40.01 ±0.011</td>
<td>52 ±0.08</td>
<td>18.45 ±0.002</td>
<td>34.8 ±0.01</td>
</tr>
<tr>
<td>% Change of Control (+ve)</td>
<td>-2.63</td>
<td>1.31</td>
<td>2.54</td>
<td>1.17</td>
<td>1.49</td>
<td>-1.19</td>
</tr>
<tr>
<td>Group6: WBF (20%)</td>
<td>7.2 ±0.03</td>
<td>13.2 ±0.07</td>
<td>37.2 ±0.07</td>
<td>51.33 ±0.06</td>
<td>18.85 ±0.006</td>
<td>35.6 ±0.09</td>
</tr>
<tr>
<td>% Change of Control (+ve)</td>
<td>-5.26</td>
<td>-3.79</td>
<td>-4.66</td>
<td>-0.14</td>
<td>3.69</td>
<td>1.08</td>
</tr>
<tr>
<td>LSD</td>
<td>0.076</td>
<td>0.11</td>
<td>0.19</td>
<td>0.09</td>
<td>0.009</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Values in each column with different letters are significantly different (P<0.05).
5.Effects of different whole barely flour proportions on Myeloperoxidase (MPO) enzyme levels in colonic tissues of treated rats.

Table (7) & Fig. (8) show the effects of different Whole Barely Flour proportions on Myeloperoxidase (MPO) enzyme levels in colonic tissues of treated rats. It could be noticed that the mean value of control (+) group was higher than control (−) group. The values were 148.2±0.07 & 131.01±0.004 pg/ml respectively, which illustrated significant difference with percent of decrease was -11.60% of control (−) group as compared to control (+) group.

Groups 4, 5, 6 for 10%WBF, 15%WBF, 20%WBF respectively, showed significant differences as compared to control (+) group. The values were 162.3±0.01, 202.6±0.05, 219.7±0.04 pg/ml for groups 5%WBF, 10%WBF, 20%WBF respectively. The percent of increase were 9.51%, 36.71%, 48.25% of the above mentioned groups respectively.

Group 3 (5%WBF) revealed significant difference with percent of decrease was -2.63% of group 3 (5%WBF) as compared control (+) group. Group 3 (5% WBF) recorded the best group for Myeloperoxidase (MPO) enzyme in treated rats when compared to control (+) group.

Myeloperoxidase (MPO) is an enzyme contained within granulocytes such as neutrophils (and to a lesser extent monocytes and macrophages) (Elson et al., 1996).
The Food and Drug Administration has included beta-glucans in natural compounds that affect the immune response. The positive impact of beta-glucans on organisms results from their immune-stimulating properties. Beta-glucans have the ability to bind to immune cells receptors, activate them, and regulate the humoral as well as cell-mediated immunity.

The anti-inflammatory activity of oat beta-glucan in the upper gastrointestinal tract was shown in our previous studies on the chronic lipopolysaccharides (LPS)-induced enteritis model (Sucheck et al., 2015; Wilczak et al., 2015).

It is a fact that neutrophils contain substantially more Myeloperoxidase (MPO) than any other cell type (Trush et al., 1994).

A high intracellular concentration of antioxidants protects MPO-containing cells from damage induced by Hypochlorous acid (HOCl) as well as other reactive oxygen species. Neutrophils use Hypochlorous acid (HOCl), and other bactericidal agents, as a weapon against pathogens that elicits an inflammatory response (Baynes and Dominiczak, 2005). It has beneficial effects on the immune system and is accepted as a booster for the immune system, and it has no toxic or side effects.

The level of Myeloperoxidase (MPO) activity is directly proportional to the neutrophil concentration in inflamed tissue and is thus an index of neutrophil infiltration and inflammation (Choudhary et al., 2001). Therefore, the measurement of MPO activity is considered a quantitative and sensitive assay for assessing acute intestinal inflammation.

Oat β-glucan in the diet may exhibit an anti-inflammatory function against colitis through inhibition of the expression of pro-inflammatory factors (Liu et al., 2015).

The percent of β-glucan in whole barley flour is 7%, thereby stimulates immunomodulatory effect and produces pro-inflammatory cytokines.
Table (7): Effects of different whole barely flour proportions on Myeloperoxidase (MPO) enzyme levels in colonic tissues of treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>MPO (pg/ml)</th>
<th>Mean ± SD</th>
<th>% Change of Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1: Control –ve</td>
<td></td>
<td>131.01f</td>
<td>±0.004</td>
<td>-11.60</td>
</tr>
<tr>
<td>Group2: Control +ve</td>
<td></td>
<td>148.2d</td>
<td>±0.07</td>
<td>0</td>
</tr>
<tr>
<td>Group3: Whole barley flour (5%)</td>
<td></td>
<td>144.3e</td>
<td>±0.08</td>
<td>-2.63</td>
</tr>
<tr>
<td>Group4: Whole barley flour (10%)</td>
<td></td>
<td>162.3c</td>
<td>±0.01</td>
<td>9.51</td>
</tr>
<tr>
<td>Group5: Whole barley flour (15%)</td>
<td></td>
<td>202.6b</td>
<td>±0.05</td>
<td>36.71</td>
</tr>
<tr>
<td>Group6: Whole Barely Flour(20%)</td>
<td></td>
<td>219.7a</td>
<td>±0.04</td>
<td>48.25</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
</tbody>
</table>

Values in each column with different letters are significantly different (P<0.05)

![Myeloperoxidase (MPO) pg/ml](chart.png)

Figure (8): Effects of different whole barley flour proportions on Myeloperoxidase (MPO) enzyme levels in colonic tissues of treated rats
Effects of different Whole Barely Flour (WBF) proportion on colon tissue cytokine levels in treated rats.

6.1. Tumor Necrosis Factor- alpha (TNF-α)

Table (8) & Fig. (9) show the effects of different Whole Barely Flour (WBF) proportions on TNF-α levels in colon tissue samples of treated rats. It could be noticed that the mean value of control (+) group was higher than control (−) group. The values were 45.9±0.06 & 31.1±0.4 pg/ml respectively, which showed significant differences with percent of decrease was -32.24% of control (−) group as compared to control (+) group.

Groups 4, 5, 6 for 10%WBF, 15%WBF, 20%WBF respectively revealed significant differences as compared to control (+) group. The mean values were 53.09±0.009, 53.8 ±0.04, and 62.5±0.06 pg/ml respectively with percent of increase were 15.66%, 17.21%, and 36.17% of above mentioned groups as compared to control (+) group.

A group 3 (5%WBF) illustrated significant difference. The percent of decrease was -5.66% of group 3 (5%WBF) as compared to control (+) group. Group 3 (5%WBF) recorded the best group for TNF-α levels in colonic tissue samples of colitis rats.

Changes in the expression of gene encoding immunomodulating cytokines and chemokines were observed simultaneously with the changes in concentration of analyzed cytokines which were the highest in the colitis group without β-glucan supplementation (CβG-). With regard to the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, our results were consistent with the data obtained by others, who showed the level of pro-inflammatory cytokines increases in animals colitis induced by exogeneous agents (Aubry et al., 2015; Chen et al., 2017).

A variety of fungal and yeast (1, 3; 1, 6-linked--D-β-glucose) and cereal (1, 3)-β-(1,4-linked-D-β-glucose) β-(1, 3) glucans have been reported to have antitumor activity (Taguchi, 1987; Kidd, 2000).

Oral uptake and biodistribution of barley or yeast β-(1→3) glucan occurred via gastrointestinal macrophages. Previously reported data (Yan et al., 1999; Cheung, 2002; Hong et al., 2003) showed that β-1, 3-glucan-mediated tumor regression requires antitumor Abs that activate complement and deposit iC3b on antitumor cells.

6.2. Interleukin- 1β (IL-1β)

Table (8) & Fig. (9) show the effects of different Whole Barely Flour (WBF) proportions on IL-1β levels in colon tissue samples of treated rats. It could be noticed that the mean value of control (+) group was higher than control (−) group. The mean value was 15.47±0.009 & 12.3±0.02 pg/ml
respectively, which revealed significant difference with percent of decrease was -20.49% of control(−) group as compared control (+) group.

Groups 4, 5, and 6 for 10%WBF, 15%WBF and 20%WBF respectively showed significant differences as compared to control (+) group. The mean values were -20.49±0.06, 25.29±0.002 and 23.3±0.09 pg/ml for 10%WBF, 15%WBF and 20%WBF respectively. The percent of increase were 37.04%, 42.86%, and 50.61% of above mentioned groups respectively. Group 3 (5%WBF) illustrated significant difference with percent of decrease was -28.25% of group 3 (5%WBF) As compared to control (+) group.

Group 3(5%WBF) recorded the best group for IL-1β levels in colonic tissue samples of colitis rats.

IL-1β is secreted mainly by the innate immune cells in response to inflammatory trigger (Snodgrass et al., 2013).

Pro-inflammation cytokines are known to play an important role in inflammation of the intestinal mucosa (Nakamura et al., 1992). Specifically increased levels of TNF-α, IL-1β, IL-6 and IL-8 have been reported in ulcerative colitis patients (O’Shea et al., 2002; Nielsen et al., 1993). IL-1β is a key cytokine involved in the activation and production of additional cytokines involved in inflammation. Akira et al. (1990) showed that IL-1 is involved in upregulating the production of IL-8, IL-6 and TNF-α.

6.3. Interleukin-10 (IL-10)

Table (8) & Fig. (9) show the effects of different whole barely flour (WBF) proportions on IL-10 levels in colon tissue samples of treated rats. It could be noticed that the mean value of control (+) group was higher than control (−) group. The mean value was 44.1±0.04 & 19.09±0.003 pg/ml respectively which revealed a significant difference of percent of decrease -56.71% of control(−) group as compared to control (+) group.

Groups 3, 4, 5, 6 for 5%WBF, 10%WBF, 15%WBF and 20%WBF respectively illustrated significant differences. The values were 13.02±0.008, 24.14±0.005, 25.29±0.002, and 28.87±0.007 pg/ml for 5%WBF, 10%WBF, 15%WBF, and 20%WBF respectively with percent of decrease were-70.48%,-45.26%, -42.65% and -34.54% for above mentioned groups respectively. Group 3 (5%WBF) recorded the best group for IL-10 levels in colonic tissue samples of colitis rats.

IL-10 is a pleiotropic cytokine involved in both cell-mediated and humoral immune responses. Furthermore, IL-10 has both anti-inflammatory and proinflammatory effects (Melgar et al., 2003). Anti-inflammatory effects include preventing colitis in severe combined immunodeficiency (SCID) mice (Asseman 1999).
In present study IL-10 mRNA increased in the DSS-only mice, similar to result reported by (Niessner & Volk, 1995) who showed increase mRNA level in crude extracts of colon biopsies from ulcerative colitis patients, and of (Kusharzik et al., 1995) who reported elevated serum levels of IL-10 in active colitis.

The inhibitory cytokines IL-10 and TGF-β in Peyer’s patches, mesenteric lymph nodes and lamina propria are involved in T-cell tolerance in the intestine. Moreover, there is a genetic association between the inhibitory cytokine IL-10 and UC, in which IL-10 participates in the down-regulation of intestinal inflammation (Abraham and Cho, 2009).

6.4. Interferon-gamma (IFN-γ)

Table (8) & Fig.(9) show the effects of different Whole Barely Flour (WBF) proportions on IFN-γ levels in colon tissue samples of treated rats. It could be noticed that the mean value of control (+) group was higher than control (−) group. The mean value was 35.35 ±0.009 & 31.92±0.04 pg/ml respectively which revealed a significant difference with percent of decrease was -9.70% of control (−) group as compared to control (+) group.

Groups 3, 4, 5 for 5%WBF, 10%WBF, 15%WBF respectively, illustrated significant differences as compared to control (+) group. The values were 32.50±0.004, 33.3±0.002, 33.8±0.008 pg/ml for 5%WBF, 10%WBF,15%WBF respectively. The percent of decrease were -8.06%, -5.80%, 33.8% of group 3, 4, 5 for above mentioned groups. Group 6 (20%WBF) showed a significant difference as compared to control (+) group. The percent of increase was 36.5% of group 6. Group 3 (5%WBF) recorded the best group for( INF-γ) levels in colonic tissue samples of colitis rats.

Both INF-γ and IL-1β are secreted during active colitis in humans and DSS induced colitis in mice from colonic tissue, INF-γ is in fact an essential factor for induction of colitis (Neurath et al., 2002; Powrie et al., 1994).

6.5. Inhibitory Subunit of NF Kappa B Alpha (IκBα)

Table (8) & Fig. (9) show the effects of different Whole Barely Flour (WBF) proportions on Inhibitory subunit of nuclear Factor kappa B alpha (IκBα) levels in colon tissue samples of treated rats. It could be noticed that the mean value of control (+) group was higher than control (−) group. The mean value was73.95±0.008 & 63.7±0.04 pg/ml respectively which showed a significant difference with percent of decrease was -13.86% of control (−) group as compared to control (+) group.

Groups 3, 4 for 5%WBF, 10%WBF revealed significant differences as compared to control (+) group. The value were 17.24±0.009 & 53.98±0.002 pg/ml respectively which revealed significant differences with percent of
decrease were -76.69%, -27.00% of groups 3, 4 for 5%WBF, 10%WBF respectively.

Groups 5, 6 for 15%WBF, 20%WBF illustrated significant differences as compared to control (+) group. The values were 56.83±0.008 & 68.8±0.03 pg/ml respectively with percent of increase were 36.71%, 48.25 % for above mentioned groups.

Group 3 (5%WBF) recorded the best group for (IkBα) levels in colonic tissue samples of colitis rats.

Characteristic histological findings in IBD is an influx of innate immune cells (neutrophils, macrophages, dendritic cells and NK cells) as well as adaptive immune cells (B cells and T cells) into the lamina propria. With activation of immune cells, there is an elevation in the TNF-α, IL-1β, IFN-γ and cytokines levels. Recent advances in genome-wide associated studies and immunological studies suggest aberration in the mucosal innate response, innate microbial sensing, autophagy and unfolded protein response are potential pathogenic pathways in IBD (Geremia et al., 2014).

Table (8): Effects of different whole barely flour (WBF) proportions on tissue cytokine levels in treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>TNF-α (pg/ml)</th>
<th>IL-1β (pg/ml)</th>
<th>IL-10 (pg/ml)</th>
<th>INF-γ (pg/ml)</th>
<th>IkBα (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Group1</td>
<td>Control (-ve)</td>
<td>31.1 ± 0.4</td>
<td>12.3 ± 0.02</td>
<td>19.09 ± 0.003</td>
<td>31.92 ± 0.006</td>
<td>63.7 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>% Change of Control (+ve)</td>
<td>-32.24 ± 0.00</td>
<td>-20.49 ± 0.00</td>
<td>-56.71 ± 0.00</td>
<td>-9.70 ± 0.00</td>
<td>-13.86 ± 0.00</td>
</tr>
<tr>
<td>Group2</td>
<td>Control (+ve)</td>
<td>45.9 ± 0.06</td>
<td>15.47 ± 0.009</td>
<td>44.1 ± 0.04</td>
<td>35.35 ± 0.009</td>
<td>73.95 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>% Change of Control (+ve)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Group3</td>
<td>WBF (5%)</td>
<td>43.3 ± 0.08</td>
<td>13.02 ± 0.008</td>
<td>13.02 ± 0.008</td>
<td>32.50 ± 0.004</td>
<td>17.24 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>% Change of Control (+ve)</td>
<td>-5.66 ± 0.00</td>
<td>-28.25 ± 0.00</td>
<td>-70.48 ± 0.00</td>
<td>-8.06 ± 0.00</td>
<td>-76.69 ± 0.00</td>
</tr>
<tr>
<td>Group4: WBF (10%)</td>
<td></td>
<td>53.09 ± 0.009</td>
<td>21.2 ± 0.06</td>
<td>24.14 ± 0.005</td>
<td>33.3 ± 0.002</td>
<td>53.98 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>% Change of Control (+ve)</td>
<td>15.66 ± 0.00</td>
<td>37.04 ± 0.00</td>
<td>-45.26 ± 0.00</td>
<td>-5.80 ± 0.00</td>
<td>-27.00 ± 0.00</td>
</tr>
</tbody>
</table>
### Table (9): Effects of different whole barely flour (WBF) proportions on tissue cytokine levels in treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>WBF (%)</th>
<th>TNF-α (pg/ml)</th>
<th>IL-1β (pg/ml)</th>
<th>IL-10 (pg/ml)</th>
<th>INF-γ (pg/ml)</th>
<th>IkBα (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 5:</td>
<td>WBF (15%)</td>
<td>53.8a ±0.04</td>
<td>25.29a ±0.002</td>
<td>25.29a ±0.002</td>
<td>33.8a ±0.008</td>
<td>56.83a ±0.008</td>
</tr>
<tr>
<td>Change of Control (+ve)</td>
<td>17.21</td>
<td>42.86</td>
<td>-42.65</td>
<td>-4.38</td>
<td>36.71</td>
<td></td>
</tr>
<tr>
<td>Group 6:</td>
<td>WBF (20%)</td>
<td>62.5a ±0.06</td>
<td>23.3a ±0.09</td>
<td>28.87b ±0.007</td>
<td>36.5a ±0.007</td>
<td>68.8b ±0.03</td>
</tr>
<tr>
<td>% Change of Control (+ve)</td>
<td>36.17</td>
<td>50.61</td>
<td>-34.54</td>
<td>3.25</td>
<td>48.25</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>0.09</td>
<td>0.09</td>
<td>0.03</td>
<td>0.011</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

Values in each column with different letters are significantly different (P<0.05).

7. Effect of different whole barley flour proportions on Histopathology of colonic tissues in treated rats

Results of histopathological examinations showed good protection of the colon mucosa with mild degree of epithelial necrosis and desquamation, and normal impact crypt and their lining goblet cells and mild inflammatory cells infiltration in colitis group3 (5% WBF) as compared to control (+) group (Photo 1). Colon of colitis model rats that treated with 10% WBF showed focal desquamation areas of the mucosal epithelium, mild inflammatory cells infiltration and submucosal edema and near to normal crypts and their lining goblet cells (Photo 2).

In regard to, Colons of colitis rats that treated with 15% WBF revealed normal appearance of the colonic crypts and normal goblet cells and very mild inflammatory infiltration and desquamated epithelium (Photo 3). While, colons of colitis rats that treated with 20% WBF showed variable degrees of desquamation of their colon mucosal epithelial linings with mild inflammatory
cells infiltration the mucosal layers accompanying normal crypts and goblet cells. Results of histopathological examination confirmed more or less the biochemical changes, (MPO), TNF-α, IL-1β, IL-10, INF-γ & IkBα indirectly, that best restoration of the histological structure achieved at 5% WBF, while at 10%, 15% & 20% WBF, although much improvement occurred but formal structure degenerations recorded in tissues.

**Photo 1:** Colon of control (-) rat (group 1) showing normal mucosal layer with normal mucosal epithelium (arrow), normal submucosal (SM) and muscular (M) layers. (H&E, X200).

**Photo 2:** Colon of colitis model rat (group 2) showing severe mucosal necrosis without cellular details, necrosis of the crypts (arrow) and presence of many shreds of necrotic desquamated cells (DCs). (H&E, X200).
Photo.3: Colon of colitis model rat that treated with WBF5% (group 3) showing good protection of the colon mucosa with moderate degree of epithelial necrosis and desquamation (short arrow), normal impacted crypts (dashed arrow) and goblet cells and mild inflammatory cells infiltration, the submucosa showing moderate edema (Ed), mild inflammatory cells (IF) and congested blood vessels (arrow). (H&E, X100).

Photo.4: Colon of colitis model rat that treated with WBF10% (group 4) showing near to normal crypts and their lining goblet cells (dashed arrow), mild inflammatory cells infiltration (IF), scars congested blood capillaries (arrow) and scattered focal areas of mucosal epithelia necrosis and desquamation (short arrow). (H&E, X200).
Fig. 5: Colon of colitis model rat that treated with 15% Whole Barley Flour (group 5) showing normal appearance of the colonic crypts (arrow) and normal goblet cells with very mild inflammatory cells infiltration and desquamated epithelium. The submucosa showing mild edema (Ed), few inflammatory cells infiltration (IF) and some congested vessels (short arrow).

Photo. 6: Colon of colitis model rat that treated with WBF20% (group 6) showing desquamation of the mucosal epithelial linings (arrow), mild inflammatory cells (IF) infiltrating the mucosal layer normal crypts and goblet cells. (H&E, X100).
Conclusion

Oral administration of DSS to male wistar rats causes a reproducible acute colitis, followed by treating with different proportions of whole barley flour. The low proportions of WBF revealed attenuating while, high proportions of WBF revealed increase inflammation to colonic tissues. Supporting of wheat bread with hull-less barley flour is healthy benefits because of inclusion the later on β-glucan have immunomodulatory effects. Health claims on the positive effect of β-glucan from barley on innate immune system and prevent colitis and colon cancer in daily consumption of 3 gram of soluble β-glucans.

Barley possesses high amount of dietary fiber (DF) with high proportion of soluble viscous components, therefore, increase barley further than 5% (in rats and monogastric, poultry & piglets) increase viscosity and decrease absorption and the therapy effect of β-glucan. In human nutrition, it could be increased the content of whole barley flour up to 20%, because of higher β-glucan is not desirable even for barley intended for monogastric feed (piglets & poultry) that cause digestive problems and reduce nutrient utilization. Addition β-(1→3),(1→4)-glucan (highly soluble fraction of barley provides thickening, emulsifying and gelation for different foods e.g. soups, sauces and ice creams etc. Support wheat bread with hulless barley flour because of inclusion the latter of β-glucan have immunomodulatory effects.
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EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2011).Scientific opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post- prandial glycaemic responses (ID 821, 824), and “digestive function” (ID 850) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal, 9(6): 2207.
EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)(2009). Scientific opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823) pursuant to Article 13(1) of Regulation (EC) No 1924/2006 on request from the European Commission. EFSA Journal, 7(9): 1254.


دراسة التغيرات البيولوجية والهستوباثولوجية والمناعية لدى الفئران البيضاء المصابة بعد إتلاف القولون الفموي الحاد بتأثير دقيق الشعر الكامل

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استاذ التغذية وعلوم الأطعمة كلية الاقتصاد المنزلي، جامعة المنوفية

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السكريات العديدة هي واحدة من أقوى المواد الداخلة في تكوين الشعر الخالي من القشرة لإظهار مضادات الالتهاب والمناعة، كان الهدف من هذه الدراسة هو تحديد ما إذا كانت التغذية عن طريق الفم بدقيق الشعر الكامل يمكن أن تخفف أو تمنع تطور التهاب القولون في فئران التجربة.

تم إحداث التهاب القولون في الفئران عن طريق المعالجة 5% (وزن/حجم) من مادة ديكستران كبريتات الصوديوم التي تم إزابتها في مياه الشرب لمدة 3 أيام متتالية. تم تغذية الفئران عن طريق النظام الغذائي الأساسي مع استباد (10%, 15%, 20%) من نشاء الذرة الداخية في تكوين النظام الغذائي الأساسي بنفس النسبة من دقيق الشعر الكامل لمدة 28 يوم للمجموعات التي أصيبت بتهاب القولون وتلتقي علاجا بتلك النسبة السابقة، في نهاية التجربة تم تقييم التلف الحاد للقولون عن طريق الاختبارات الهستوباثولوجية وقياس طول القولون، وزن الأعضاء الداخلية (القولون-الطحال-الكبد-القلب-الرئتين) والقياسات العينية بقيم درجات (نقص الوزن-وجود دم متخفى بالبراز-قوام البراز)؛ وكذلك تم تقييم الالتهاب من خلال التغيرات في مستويات عامل نخر الورم-ألفا، ونشاط إنزيم الميلوبروكسيديز، وإنترلوكين-1ب، وإنترلوكين-10، إنترفرون-جاما، والبروتين الفرعي المناعي للعامل النووي (مانع تنشيط نسخ الجينات) في سيتوبلازم الخمية لعينات أنسجة القولون، أدت نسبة المنخفضة من دقيق الشعر (15%) إلى منع تقصير القولون، كما أدت هذه النسبة أيضا إلى تخفيف أعراض التهاب القولون الحاد في المجموعة التي تلقى علاجا بنسبة 5% بالمقارنة بالمجموعات الأخرى، وكذلك مع نشاط إنزيم الميلوبروكسيديز وتحسين في كرات الدم الحمراء والهيموغلوبين والهيماتوكريت، ونوعية جميرة الدم، ونوعية تركز الهيموغلوبين في كرية الدم، وكرات الدم البيضاء، والخلايا المناعية عديدة النواة، والخلايا الليمفاوية، والخلايا المناعية وحيدة النواة، والمصفات الدموية، بالإضافة إلى ذلك تم تقليل الضرر الحاد في الأنسجة من خلال التشريح الهستوباثولوجي، مستوي عامل نخر الورم-ألفا، وإنترلوكين-1ب، وإنترلوكين-10، وإنترفرون-جاما، والبروتين الفرعي المناعي للعامل النووي انخفضت بشكل ملحوظ وربطت بدرجات الإلتهاب وأعراض المرض.