EFFECTS OF INTERMITTENT FASTING ON BODY WEIGHT, BIOCHEMICAL PARAMETERS AND HISTOPATHOLOGICAL PICTURE OF COLON IN RATS WITH ULCERATIVE COLITIS

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**ABSTRACT:**
Intermittent fasting (IF) is a promising strategy among different approaches of fasting. It has ability to cope up with different diseases as ulcerative colitis (UC). This study was aimed to explore effects of IF of rats with UC on body weight, blood chemistry and histological picture of colon. Thirty-five mature rats weighing 225±5 g BW were used. Rats were distributed into 5 equal groups, Group1: negative control, group 2: positive control with UC and groups 3, 4 and 5: with UC and were fasted for 12, 16 and 20 hr, respectively. Feed intake, body weight gain and feed efficiency ratio were calculated. Blood samples were collected for determination of activities of serum liver and kidney functions as well as lipid profile. Proinflammatory cytokines i.e. interleukin 1 beta (IL1β), IL6 and IL8 were measured. Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) were estimated. In liver homogenate, reduced glutathione (GSH), malondialdehyde (MDA) and lactate dehydrogenase (LDH) were measured. Histopathological examination of large intestine was also done. The results revealed that IF of rats with UC significantly decreased serum levels of liver and kidney functions, lipid profile, IL1β, IL6 and IL8. Activities of GPx, SOD and CAT were increased. Liver tissue GSH was increased, but MDA and LDH were decreased. Histopathology showed that IF mitigated inflammatory lesions of large intestine. It could be concluded that intermittent fasting plays an important role for weight loss and reduction of gut inflammation.

**Key Words:** Intermittent fasting, Ulcerative colitis, Body weight, Biochemical parameters, Histopathology

**INTRODUCTION:**
Intermittent fasting is a promising strategy among different approaches of fasting such as caloric restriction (CR) and dietary restriction (DR). It is proved to be the most fruitful approach for its ability to cope up with different diseases such as cancer, diabetes, antioxidant stress, ulcerative colitis, cardiovascular diseases, renal diseases and hypertension (**Mattson et al., 2017**). There are four different
methods of doing fasting i.e. time-restricted fasting (TRF); alternate-day fasting (ADF); twice-a-week method and Eat: Stop: Eat method. The method of fasting used in this study was TRF i.e. fasting for 12 hr, 16 hr or 20 hr.

Intermittent fasting of rats with UC exerts antioxidant effects by increasing the synthesis of reduced glutathione which serve as the most important protective systems against oxidative stress and lipid peroxidation (Rocha, et al. 2002). IF is known to lower levels of systemic inflammation and pro-inflammatory cytokines (Ostroukhova et al., 2012).

Ulcerative colitis is an idiopathic inflammatory bowel disease that causes irritation, inflammation, damage, and ulcers in the mucosa of the large intestine and rectum. Multiple factors, such as genetic background, environmental and luminal factors, and mucosal immune dysregulation, have been suggested to contribute to UC pathogenesis (Ng et al., 2013). Ulcerative colitis is one of two major forms of inflammatory bowel disease and is characterized by mucosal inflammation initiating in the rectum and extending proximally in the colon in a continuous fashion. By contrast, inflammation in Crohn’s disease (CD), the other type of inflammatory bowel disease, demonstrates patchy lesions that are potentially scattered anywhere in the gastrointestinal tract (Magro, 2017).

In recent year, IF became a popular new paradigm for causing weight loss and reducing gut inflammation, as well as numerous health benefits (Khalifa et al., 2021). The beneficial effects of IF of rats are still need further investigations. Therefore, the present study was aimed to explore effects of intermittent fasting of rats with acetic acid-induced ulcerative colitis on body weight, blood chemistry and histological picture of colon.

MATERIAL and METHODS:

Materials:

Acetic acid (AA): It is a liquid substance that can induce ulcerative colitis. Acetic acid is a simple monocarboxylic acid containing two carbon atoms. The molecular chemical formula of acetic acid is: CH₃COOH. It was obtained as 5% solution (V/V) backed in 500 ml bottles from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt.

Basal diet: Basal diet was prepared according to the method of Reeves et al., (1993). It is consisted of 20% protein (casein), 10% carbohydrate, 4% fat (corn oil), 2% choline chloride, 1% vitamin mixture, 3.5% salt mixture and 5% fibers. The remainder was corn starch up to 100%.

Rats: A total number of thirty-five rats of Sprague Dawley strain weighing about 225±5 g body weight and 8 months age were used in this
study. Rats were purchased from the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

Methods:

Induction of ulcerative colitis: Rats were administered via intrarectal instillation of acetic acid (AA) at 24-hr intervals for 7 days with a plastic catheter. One ml of 3% AA was instilled into the anus then rats were maintained in the Trendelenburg position for 15 minutes to induce UC (Wang et al., 2019).

Experiment and grouping of rats: Thirty-five Sprague Dawley strain rats were housed in well-ventilated cages at room temperature (25±3 °C), 50% ± 5% humidity with a 12 hr dark/light cycle. Rats were adapted for one week on AIN-93 basal diet, and received water ad libitum. The experiment was carried out according to the guidelines of the Institutional Animal Care and Use Committee, (IACUC), Cairo University. Rats were distributed into five groups which were group 1: negative (normal) control group basal diet and water were provided ad libitum; group 2: positive control with acetic acid-induced ulcerative colitis (UC). Groups 3, 4 and 5 were with UC and fasted for 12, 16 and 20 hr, respectively. The rats were weighed at the beginning and end of the dietary period. Daily feed intake (FI) was recorded day after day throughout the experimental period (3 weeks). Determination of body weight gain per cent (BWG%) and feed efficiency ratio (FER) were assessed according to the method described by Chapman et al., (1959) using the following equations:

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

\[
\text{Feed efficiency ratio (FER)} = \frac{\text{Weight gain (g)}}{\text{Feed consumed (g)}}
\]

At the end of the experimental period (3 weeks), the rats were fasted overnight and anesthetized by sodium pentobarbital (Nesdonal®). Blood samples were collected from orbital plexus of eye and centrifuged at 3000 rpm for 15 minutes to obtain clear serum for biochemical analysis. Livers were taken out for preparation of liver homogenates. Large intestines (colons) were dissected out and kept in 10% neutral buffered formalin solution then processed for histopathological examination.

Biochemical Parameters:

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to the method of Bergmeyer et al., (1978). Serum uric acid and creatinine levels were estimated as described by Lorentz and Brendt (1967) and Agbafor et al., (2015), respectively. Serum total cholesterol (TC) was calorimetrically determined according to Allain et al., (1974) and triglycerides (TG) according to Wahlefeld (1974). Interleukin 1 beta (IL1β), IL6 and IL8 were estimated according to Sutton et al., (2006). Activities of serum glutathione peroxidase (GPx), superoxide dismutase
(SOD) and catalase (CAT) enzymes were determined using a spectrophotometer according to Paglia and Valentine (1967), Nishikimi et al., (1972) and Aebi (1984), respectively.

Preparation of liver homogenates:

One gram of the liver was collected from each rat, washed in ice-cooled 0.9% NaCl solution and homogenized in ice-cooled 10 ml of 1.15% potassium chloride solution in 50 mMol potassium phosphate buffer solution (pH 7.4) to get 10% (W/V) hepatic homogenate. The homogenates were centrifuged at 9000 rpm for 15 minutes and the supernatants were utilized to determine reduced glutathione (GSH) (Jollow et al., 1974); malondialdehyde (MDA), a marker of lipid peroxidation, (Ohkawa et al., 1979), and lactate dehydrogenase (LDH) (Hendrich and Hule, 1967).

Histopathology:

The colon was dissected out, rinsed with saline and preserved in formalin solution. The preserved colon was trimmed, washed and dehydrated in ascending grades of alcohol (70% to 100%). Small amount of colon tissue was used for sectioning and staining. The specimens were stained with Hematoxylin and Eosin (H&E) and examined microscopically according to Bancroft and Stevens (1977).

Statistical analysis:

Data were expressed as means ± S.E.M and statistical analysis was carried using one way ANOVA test with a computerized SPSS program. Significance was considered at \( P < 0.05 \) (Snedecor and Cochran, 1986).

RESULTS:

Intermittent fasting for 12, 16 and 20 hours of rats with acetic acid-induced ulcerative colitis significantly decreased daily feed intake, BWG % and FER as compared to the positive control group as recorded in Table (1).

Table (1): Effect of intermittent fasting on FI, BWG % and FER in rats with ulcerative colitis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>FI (g/day)</th>
<th>BWG (%)</th>
<th>FER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td></td>
<td>23.0</td>
<td>11.69±0.56b</td>
<td>0.080±0.0012</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td></td>
<td>20.0</td>
<td>4.74±0.41a</td>
<td>0.041±0.0021</td>
</tr>
<tr>
<td>Fasting (12 hr)</td>
<td></td>
<td>18.0</td>
<td>4.66±0.20a</td>
<td>0.040±0.0011</td>
</tr>
<tr>
<td>Fasting (16 hr)</td>
<td></td>
<td>15.5</td>
<td>3.69±0.25a</td>
<td>0.037±0.0022</td>
</tr>
<tr>
<td>Fasting (20 hr)</td>
<td></td>
<td>13.3</td>
<td>1.89±0.52a</td>
<td>0.020±0.0066</td>
</tr>
</tbody>
</table>

Means with different superscript letters in the same column are significant at \( P < 0.05 \)

The results revealed that in positive control (+ve) group, there was a significant \( P < 0.05 \) increase in both serum levels of AST and ALT as compared to the control negative (- ve) group. Intermittent fasting of rats with UC significantly decreased serum levels of both AST and ALT as compared to control (+ve) group (Table 2).
Table (2): Effect of intermittent fasting on liver functions of rats with experimentally-induced ulcerative colitis by acetic acid.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>AST µ/L</th>
<th>ALT µ/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td></td>
<td>40.57±1.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.68±0.66&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td></td>
<td>85.28±2.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.57±1.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fasting (12 hr)</td>
<td></td>
<td>49.17±2.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.00±0.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fasting (16 hr)</td>
<td></td>
<td>45.22±1.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.14±0.91&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fasting (20 hr)</td>
<td></td>
<td>41.71±1.99&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.14±0.79&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with different superscript letters in the same column are significant at (P < 0.05)

There was a significant increase in both uric acid and creatinine serum levels in the control positive (+ve) group as compared to the negative control group. The intermittent fasting of rats with ulcerative colitis significantly (P < 0.05) decreased both uric acid and creatinine serum levels as compared to the positive control group (Table 3).

Table (3): Effect of intermittent fasting on kidney functions of rats with experimentally-induced ulcerative colitis

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>Uric acid mg/dL</th>
<th>Creatinine mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td></td>
<td>33.14±0.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.74±0.076&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td></td>
<td>62.42±1.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.42±1.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fasting (12Hr)</td>
<td></td>
<td>49.71±1.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.14±1.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fasting (16Hr)</td>
<td></td>
<td>37.14±2.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.94±0.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fasting (20Hr)</td>
<td></td>
<td>34.42±1.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.14±0.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with different superscript letters in the same column are significant at (P < 0.05)

The results showed that serum levels of TC and TG were significantly reduced by IF of rats with UC, as compared to the positive control as shown in Fig. (1).

Fig. (1): Effect of IF on serum levels of TC and TG in rats with UC.
As shown in Table (4), the rats with acetic acid-induced UC in the positive control (+ve) group had significant \((P < 0.05)\) increases of Interleukin-1 beta (IL-1\(\beta\)), IL6 and IL-8, as compared to the negative control (-ve) group. Intermittent fasting of rats for 12, 16 and 20 hr significantly \((P < 0.05)\) decreased IL-1\(\beta\), IL6 and IL-8, as compared to the positive control (+ve) group.

### Table (4): Effect of intermittent fasting on Interleukin-1 beta, Interleukin-6 and Interleukin-8 in rats with acetic acid induced UC.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>IL-1(\beta) (Pg/ml)</th>
<th>IL-6 (Pg/ml)</th>
<th>IL-8 (Pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td></td>
<td>56.28±2.20(^a)</td>
<td>54.08±2.20(^#)</td>
<td>75.57±2.63(^e)</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td></td>
<td>89.57±2.39(^a)</td>
<td>69.16±2.39(^#)</td>
<td>85.14±6.52(^e)</td>
</tr>
<tr>
<td>Fasting (12 hr)</td>
<td></td>
<td>66.42±3.80(^#)</td>
<td>56.42±1.80(^#)</td>
<td>79.71±4.91(^#)</td>
</tr>
<tr>
<td>Fasting (16 hr)</td>
<td></td>
<td>46.00±4.76(^#)</td>
<td>50.00±2.76(^#)</td>
<td>65.80±2.74(^#)</td>
</tr>
<tr>
<td>Fasting (20 hr)</td>
<td></td>
<td>35.42±2.99(^#)</td>
<td>45.42±1.19(^#)</td>
<td>62.14±3.18(^#)</td>
</tr>
</tbody>
</table>

Means with different superscript letters in the same column are significant at \((P < 0.05)\).

### Table (5): Effect of intermittent fasting on serum GPx, SOD and CAT antioxidant enzymes in rats with ulcerative colitis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>GPx (mg/dL)</th>
<th>SOD (mg/dL)</th>
<th>CAT (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td></td>
<td>85.12±0.22(^a)</td>
<td>76.56±0.06(^#)</td>
<td>18.05±0.40(^#)</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td></td>
<td>69.85±0.34(^a)</td>
<td>45.42±1.13(^#)</td>
<td>10.0±1.12(^#)</td>
</tr>
<tr>
<td>Fasting (12 hr)</td>
<td></td>
<td>77.71±0.28(^#)</td>
<td>57.14±1.26(^#)</td>
<td>12.94±0.63(^#)</td>
</tr>
<tr>
<td>Fasting (16 hr)</td>
<td></td>
<td>78.14±0.80(^#)</td>
<td>58.94±0.63(^#)</td>
<td>13.55±0.63(^#)</td>
</tr>
<tr>
<td>Fasting (20 hr)</td>
<td></td>
<td>79.02±0.42(^#)</td>
<td>59.14±0.40(^#)</td>
<td>14.77±0.43(^#)</td>
</tr>
</tbody>
</table>

Means with different superscript letters in the same column are significant at \((P < 0.05)\).

As recorded in Table (6) the results indicated that IF of rats with acetic acid-induced UC increased hepatic reduced glutathione, but decreased malondialdehyde, a marker of lipid peroxidation, and lactate dehydrogenase, as compared to the positive control group.
Table (6): Effect of intermittent fasting on liver reduced glutathione, malondialdehyde and lactate dehydrogenase enzyme in rats with ulcerative colitis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>GSH (nmol/mg protein)</th>
<th>MDA (nmol/mg protein)</th>
<th>LDH (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (−ve)</td>
<td>45.12±0.32*</td>
<td>57.57±0.36*</td>
<td>14.17±0.49*</td>
</tr>
<tr>
<td></td>
<td>Control (+ve)</td>
<td>29.85±0.34*</td>
<td>76.14±1.88*</td>
<td>18.28±0.52*</td>
</tr>
<tr>
<td></td>
<td>Fasting (12 hr)</td>
<td>34.71±0.28*</td>
<td>70.72±1.98*</td>
<td>12.00±0.72*</td>
</tr>
<tr>
<td></td>
<td>Fasting (16 hr)</td>
<td>39.14±0.30*</td>
<td>68.85±1.56*</td>
<td>10.71±0.28*</td>
</tr>
<tr>
<td></td>
<td>Fasting (20 hr)</td>
<td>42.28±0.42*</td>
<td>60.71±0.92*</td>
<td>9.02±0.42*</td>
</tr>
</tbody>
</table>

Means with different superscript letters in the same column are significant at $P < 0.05$

Histopathology:

The histopathology findings of large intestines of rats were illustrated in Fig. (2)

Fig. (2): Photomicrograph of rat colon of the different collected groups: (a): Negative control group showing normal histological structure of mucosa and submucosa of colon. (b): Positive control group showing heavy inflammatory cells infiltration in the lamina propria and submucosa. (c) Group of fasted rats for 12 hr showing moderate focal area of inflammatory cells infiltration in the submucosal layer. (d) Group of fasted rats for 16 hr showing few foci of inflammatory cells aggregations in the lamina propria. (e) Group of fasted rats for 20 hr showing apparently normal histological architecture of colon mucosa and submucosa. (f) Chart showing colon histopathologic scores of the different groups.
DISCUSSION:

The purpose of the current study was to explore effects of IF of rats with UC on body weight, blood chemistry and histological picture of colon. The present results revealed that IF decreased food intake (FI), body weight gain (BWG) and feed efficiency ratio (FER), causing weight loss. These findings are in agreement with those reported by López-Varela et al., (1995); Mattson et al., (2017) and Welton et al., (2020). The previous authors concluded that IF shows promise as a primary care intervention and treatment of obesity and it can play a role in weight loss. In animal models, IF improves insulin sensitivity, prevents obesity caused by a high-fat diet, and ameliorates diabetic retinopathy. Furthermore, other studies involving overweight or obese adults have shown that IF is as effective for weight loss (Fitzgerald et al., 2018).

The results cleared that IF increased activities of serum antioxidant enzymes in rats with acetic acid-induced UC. These findings are similar to those reported by Mueller et al., (2013) and Zahang et al., (2020) who concluded that IF is an effective treatment of acetic acid-induced ulcerative colitis and suppress the inflammatory responses and oxidative stress in gut. It was reported that IBD is associated with an imbalance between increased ROS and decreased antioxidant activity, which may explain, at least in part, many of the clinical pathophysiological features of both CD and UC patients (Balmus et al., 2016).

Concerning liver function, it was found that IF of rats with UC significantly reduced serum levels of elevated AST and ALT enzymes. This is partially similar to that reported by Pirmadah et al., (2020) in patients who mentioned that IF might positively affect liver function in diseased patients.

IF of rats with UC also significantly decreased serum concentrations of UA and Cr. This result partially agree with that recorded by Rocha et al., (2002) in rats with hepatocarcinogenesis induced by dimethylamine. It was reported that IF decreased levels of proinflammatory cytokines Interleukin-1 beta (IL-1β), Interleukin-6 (IL6) and Interleukin-8 (IL-8) in rats with UC. This findings are similar to those obtained by Kowluru and Odenbach (2004).

Regarding antioxidant enzymes, intermittent fasting increased serum GPx, SOD and CAT antioxidant enzymes in rats with ulcerative colitis. The current results cleared that IF decreased serum TC and TC in rats with UC. This is similar to that reported by Meng et al., (2020). It was found that IF increased liver GSH, but decreased MDA and LDH in rats with UC. This finding is partially similar to that reported by Ozdemir et al., (2005) in diabetic patients.

Histopathological examination of colons showed that IF decreased inflammatory cells infiltration and ameliorated UC in rats. This finding
was similar to that obtained by Zhang et al., (2020) who concluded that IF of rats with UC mitigated inflammatory cells infiltration of colon and suppressed ulcerative colitis.

CONCLUSION:

It could be concluded that IF of rats with UC produces weight loss, improves liver and kidney functions, reduces proinflammatory cytokines and increases antioxidant enzymes and reduced glutathione, and decreased malondialdehyde. It protects colon against ulcerative colitis and oxidative damage.

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تم فحص نسيج القولون هستولوجيا. وقد أظهرت النتائج أن الصيام المتقطع في الفئران المصابة بالتهاب القولون التقرحي أدى إلى انخفاض معنوي في مستويات سيرم كل من وظائف الكبد والكلي وصورة الدهون. وكذلك السيتوكينات المسببة للالتهابات. بينما تمت زيادة أنشطة إنزيمات CAT و SOD و GPx. إضافة إلى ذلك اوضحت النتائج وجود زيادة معنوية في مستوى الجلوتاثيون بيرهاكسيديز في أنسجة الكبد، ونقص معنوي في مستويات (MDA) و (LDH).

وقد أظهر الفحص الهستوباثولوجي للقولون أختفاء علامات الالتهاب. ويمكن الاستنتاج أن الصيام المتقطع يلعب دورًا مهمًا في إنقاص الوزن وتقليل التهاب القولون.