Effects of Age and Sex on the Healing of Acetic-Acid Induced Ulcerative Colitis in Adult Wistar Rats

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Authors’ contributions

This work was carried out in collaboration among all authors. Author SFI designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors WOA and ATA managed the analyses of the study. Authors BSO and VAO managed the literature search. All authors read and approved the final manuscript.

ABSTRACT

Aims: Ulcerative colitis is a disease of the bowel that occurs in all ages and affects both males and females. This research study was designed to investigate the effect of age and sex on the healing of colitis in rats.

Methodology: Twenty-eight rats were randomly distributed into four groups of seven animals per group; adult male rats, mid age male rats, adult female rats and mid age female rats. Mid age and adult Wistar rats were 7-8 weeks and 14 weeks old respectively. Colitis was induced through a single intra-colonic instillation of 7% acetic acid (1mL/100g body weight) and allowed to heal for 14 days. Blood samples were obtained for analysis. Colon samples were also obtained for histomorphological study and biochemical assays (Myeloperoxidase activities, Superoxide dismutase, Glutathione, Catalase and Malondialdehyde) levels.

Results: There was no significant difference in Malondialdehyde concentration, catalase, Superoxide dismutase, Platelet Distribution Width, Platelet Count, Basophil cell numbers, Eosinophil cell numbers, platelet cells, Mean Platelet Volume, Mean Cell Volume and...
white blood cells across the groups. The Glutathione concentration in mid age male rats was significantly increased when compared with adult male rats. The haemoglobin, Lymphocytes and Mean Cell Haemoglobin levels were increased while neutrophils and monocyte levels were decreased in the younger female rats. The histomorphological study revealed poorly preserved surface epithelia layer of the colon in adult male rats while mid age male and female rats showed moderately preserved surface epithelia layer, adult female rats showed normal surface epithelia layer.

Conclusion: Mid age rats heal faster than adult rats while in terms of sex, female rats tends to heal faster than male rats.

Keywords: Age; sex; colitis; healing.

1. INTRODUCTION

Colitis is the inflammation of the large intestine resulting in excruciating abdominal pain and discomfort in the body. Sometimes this abdominal pain may be mild or acute and persistent or re-occurring [1,2]. Ulcerative Colitis (UC) is often mistaken for Crohn’s Disease (CD) though there are some differences between them. Both ulcerative colitis and Crohn’s disease are the two main types of Inflammatory Bowel Diseases (IBD) and complex diseases of unknown etiology and pathogenesis in man as elucidated by Shivashankar et al. [3].

About 2.1 million persons in Europe are affected by Inflammatory Bowel Diseases (IBD). It is lower in Central and Eastern Europe, South America, Africa but high in the developed countries like United Kingdom (UK), United States of America (USA) and Scandinavian countries [4]. It is also reported that 1.5% - 28% of persons having IBD have relatives either the parent or sibling with such disease [4,5]. Burisch, 2014 [6], also pointed out that the rate of ulcerative colitis has gradually increased exponentially in Asia in the last 20 years. Correspondingly, IBD rose from 0.2/100,000 to 1.2 per 100,000 persons in India [7].

Johnston and Logan [8] reported that both diseases occur in ages 15-35, from teenage years to young adulthood and affect both males and females. While ulcerative colitis strictly affects the colon or large intestines, Crohn’s disease affects the digestive tract which is from the mouth to the anus. Also, there may be a continuous inflammation of the colon in ulcerative colitis but in Crohn’s disease, there is a mixture of both the healthy parts and the inflamed parts. Moreover, only the innermost lining of the colon is affected in ulcerative colitis while all the layers of the walls of the bowel are affected by Crohn’s disease, [2,3].

Ulcerative colitis is an inflammatory disease caused by food poisoning from bacteria, viruses and parasites and this can be noticed from severe cramping or pain in the abdomen, loss of weight, fever, diarrhea with blood stained stool, ballooning of the abdomen and vomiting, [1,2,3].

Both age and sex play a vital role in ulcerative colitis as accounted by Lakatos [5] who affirmed that ulcerative colitis particularly affects people from age 15 years to 35 years with 7-20% being children and 60-85% are persons under 40 years [5]. In the USA, people that are above 65 years of age have incidences of ulcerative colitis which are about 8 x 10^5 times higher rate in men than women [5]. Katz and Pardi [9-12] supported that people with inflammatory bowel disease are similar in ulcerative colitis and Crohn’s disease [10]. Generally, before the age of 45 years both males and females show similar occurrence of ulcerative colitis but above age 45 years, females have lower risk of incidence than males postulated Shah et al. [12] and Shah et al. [13]. It is observed that in the United States, ulcerative colitis is more frequent in males while Crohn’s disease is more prevalent in females [4, 5, 10,14].

The main cause of inflammable disease has not been known for certain but people with this disease may have abnormal immune system and are more likely to also have poor health conditions such as cancer, liver disease, respiratory disease, arthritis and cardiovascular disease [9]. This research study was designed to investigate the extent to which age and sex have on the healing of ulcerative colitis.

2. MATERIALS AND METHODS

2.1 Animals Care and Management

A total of twenty eight (28) Wistar rats were randomly distributed into four groups of seven
animals per group. Animals aged between 7-8 weeks and 14 weeks were classified as mid age rats and adult rats respectively as reported by Sengupta, [15]. They were fed with pelletized feed Mash \textit{ad libitum}, provided water through drinking trough and kept under 12 hour light and 12 hour darkness at room temperature. They were kept in polyvinyl wire mesh cages in the animal house of Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

The study was conducted in line with the guidelines of National Institute of Health (NIH) for the use of the laboratory rats (NIH publication No. 8523, revised 1985).

2.2 Experimental Design

The animals were randomly grouped into four groups containing five animals each:

Group A – Were adult male rats of 14 weeks old
Group B – Were mid-age male rats of 7 to 8 weeks old
Group C – Were adult female of 14 weeks old
Group D – Were mid-age female rats of 7 to 8 weeks old.

Colitis was induced in all the animals and allowed to heal for fourteen days.

2.3 Experimental Procedure

After the rats were weighed and randomly grouped into: Group A (Adult male rats), Group B (Mid age male rats), Group C (Adult female rats) and Group D (Mid age female rats, 7% acetic acid was used to induce colitis in all the rats and allow to heal for 14 days.

All the animals were fasted for 24 hours (by removing the feeds and all the wood shavings from the cages); colitis was then induced through a single intra-colonic instillation of 7% acetic acid (1 ml/100 g of body weight).

Sacrifice, Blood collection and assessment of colon: All animals were sacrificed by cervical dislocation at the end of two weeks post colitis induction. After sacrificing the animals through cervical dislocation, blood samples (2mL) were obtained from the animal through cardiac puncture and the blood sample of each rat was collected into Ethylene diamine tetra acetic acid (EDTA) bottle.

The distal colon (6cm from the anus) was also dissected out and opened longitudinally, washed to remove luminal content with ice-cold normal saline to preserve the living cells. The thickness of the colon wall was measured with meter ruler in mm.

2.4 Determination of Change in Weight of the Animals

The change in weight of the animal was obtained by the following formula:

\[
\text{Weight gain} = (\text{final weight} - \text{initial weight (before colitis induction)})
\]

Determination of hematological parameters: The collected blood samples were used for the determination of red cell count, packed cell volume, hemoglobin concentration, white blood cell count and differentials count using Mindray auto haematological analyzer (Shenzhen Mindray Bio-Medical electronics, Co., LTD, China).

The assessment of diarrhea, macroscopic ulcer scores and assessment of Colitis: Diarrhea (loose, watery stools, stool with visible blood) study and Macroscopic damage score were done following the previous methods of Masonobi et al., 2002 and Ige et al.,2020 respectively, [16,17].

The distal colon tissues section dissected out were weighed, then divided into two portions; a portion for histological analysis while the large portion of colonic tissue homogenized in phosphate buffer solution (PBS) and then centrifuged at a speed of 16,000rpm at 4°C for 20 minutes. The resultant supernatants were kept at 4°C for biochemical assay.

Biochemical Assays: Colonic Tumor Necrosis Factor-alpha (TNFα) and Myeloperoxidase (MPO) activities were determined using Enzyme-Linked Immunosorbent Assay (ELISA) kit (with Elabsience Biotechnology Inc., U.S.A).

Additionally, colonic Superoxide dismutase (SOD), Glutathione (GSH), Catalase (CAT) and Malondialdehyde (MDA) levels were determined as reported by Ige et al., [18], Ellman [19], Zhou and Kang [20], and Vashney and Kale, [21], respectively.

Histopathological assessment of Colonic tissue: Histopathological assessment of colonic tissue was done as described in a previous study [22], the harvested colon specimens were rinsed in ice-cold normal saline and then fixed in 10 %
formalin. The specimens were then processed through graded alcohols into paraffin wax, sections of each specimen from paraffin embedded were serially stained with a modified Masson trichrome stain.

2.5 Statistical Analysis

Results were presented as mean ± standard error of mean (SEM). Data were analyzed with Graph Pad Prism version 5 software, using a one-way analysis of variance (ANOVA) followed by unpaired Student’s test. Significant difference level was set at P<0.05.

3. RESULTS

3.1 Body Weight, Colonic Weight, Colonic Thickness, Ulcer Score and Diarrhea Score in Male and Female Colitic Rats

The body weights gain of adult male, mid age male and adult female rats were significantly decreased (P < 0.0001) (while there was no change in body weight of mid age female rats, Fig. 1.

There was no significant difference in colonic weight (P = 0.36) and colonic thickness (P = 0.85) across the groups, Table 1. There was presence of ulceration in the adult males and mid age males rats colon, while the adult females and mid age females show absence of ulcerations, Table 1. There was presence of loose stools in the adult male and mid age male rats, however, adult female and mid age female rats stool was normal, Table 1.

3.2 Effect of Age and Sex on Oxidative Stress Markers in Male and Female Colitic Rats

There was no significant difference (P = 0.201) in malondialdehyde (MDA) concentration, across the groups, Fig. 2.

There was no significant difference in catalase activities (P= 0.68) across the groups, Fig. 3.

There was no significant difference in superoxide dismutase (P = 0.48) activities across the groups, Fig. 4.

Reduced glutathione (GSH) concentration in mid age male rats was significantly higher than that of adult male rats (P = 0.02), Fig. 5.

3.3 Effect of Age and Sex on Myeloperoxidase Activity in Male and Female Colitic Rats

There was no significant difference in Myeloperoxidase (MPO) activities in mid age female rats when compared with mid age male rats (P =0.11)and adult female rats (P = 0.51).

![Fig. 1. Effects of age and sex on changes in body weight in male and female colitic rats](image-url)
Table 1. The effects of age and sex on colonic weight, colonic thickness, ulcer scores and diarrhea score in male and female colitic rats

<table>
<thead>
<tr>
<th></th>
<th>Colon weight (g)</th>
<th>Colon thickness (mm)</th>
<th>Ulcer scores</th>
<th>Diarrhea score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Male</td>
<td>0.57 ± 0.02</td>
<td>0.11 ± 0.00</td>
<td>0.80 ± 0.80</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td>Mid age male</td>
<td>0.73 ± 0.13</td>
<td>0.11 ± 0.00</td>
<td>1.80 ± 0.92</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td>Adult females</td>
<td>0.58 ± 0.05</td>
<td>0.10 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Mid age females</td>
<td>0.72 ± 0.16</td>
<td>0.10 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

Fig. 2. Effects of age and sex on colonic malondialdehyde concentration in male and female colitic rats

Fig. 3. Effects of age and sex on colonic catalase activity in male and female colitic rats

3.4 Hematological Indices in Male and Female Colitic Rats

Table 2 below shows alteration in hematological indices in adult male rats, mid age male rats, adult female rats and mid age female rats after induction of colitis.

3.5 Effect of Age and Sex on histological section of male and female colitic rats

The fig. 7 shows the histological sections of rats 14th day post colitis induction.
4. DISCUSSION

In this study there was a significant decrease in the body weights of adult male, mid age male and adult female rats 14th day post colitis induction as indicated in Fig. 1, but there was no change in body weight of mid age female rats. This is similar to the findings of Bábičková et al. [23] who discovered that both male and female Dextran Sodium Sulphate (DSS) - induced colitis mice had weight loss but the female mice only began to regain weight after the removal of DSS (regenerative phase). Both adult male and mid age male rats have a higher decrease in body weight than the female adult rats which is congruent with the results of Resta-Lenert et al. [24] and Bábičková et al. [23]. Human clinical researches also observed that male patients with ulcerative colitis had weight loss while the female patients do not suffer from weight loss, [25,26]. Bábičková et al. [23] also reported progressive loss of body weight in male mice compared with the female mice. Just like the effect recorded in this study, the female mice in the study by Babiekova et al. [27-30] recovered faster. The difference may have been accounted for by the effect of progesterone [30].

Fig. 4. Effects of age and sex on colonic superoxide dismutase activity in male and female colitic rats

Fig. 5. Effects of age and sex on colonic Reduced Glutathione concentration in male and female colitic rats

*significant difference (P = 0.02) when compared with adult male rats
Fig. 6. Effects of age and sex on colonic Myeloperoxidase activity in male and female colitic rats

*significant difference (P = 0.03) when compared with mid age male rats and adult female rats

Table 2. The effects of age and sex on hematological indices in male and female colitic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Adult male</th>
<th>Mid age male</th>
<th>Adult female</th>
<th>Mid age female</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (x10^9/L)</td>
<td>6.36 ± 0.27</td>
<td>5.12 ± 0.39(^a)</td>
<td>5.29 ± 0.32</td>
<td>6.31 ± 0.12(^b)</td>
</tr>
<tr>
<td>WBC (x10^9/L)</td>
<td>3.24 ± 0.70</td>
<td>5.25 ± 1.18</td>
<td>3.55 ± 0.61</td>
<td>4.34 ± 0.69</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>12.33 ± 0.36</td>
<td>11.05 ± 0.44</td>
<td>10.55 ± 0.73</td>
<td>12.73 ± 0.29(^c)</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>44.03 ± 0.88</td>
<td>36.18 ± 1.80(^d)</td>
<td>35.05 ± 3.21</td>
<td>39.53 ± 1.31(^e)</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>67.18 ± 3.83</td>
<td>63.88 ± 1.96</td>
<td>61.43 ± 0.43</td>
<td>63.00 ± 1.25</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18.75 ± 0.55</td>
<td>18.95 ± 0.25</td>
<td>19.48 ± 0.33</td>
<td>19.95 ± 0.21(^f)</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>28.75 ± 0.35</td>
<td>31.28 ± 0.66(^g)</td>
<td>31.70 ± 0.50</td>
<td>32.58 ± 0.39(^h)</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>17.28 ± 2.12</td>
<td>11.43 ± 2.40</td>
<td>8.53 ± 0.13</td>
<td>6.20 ± 1.30(^i)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>64.43 ± 2.36</td>
<td>68.38 ± 5.00</td>
<td>67.40 ± 0.37</td>
<td>77.15 ± 1.74(^j)</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>9.73 ± 3.66</td>
<td>12.78 ± 7.17</td>
<td>21.78 ± 0.68</td>
<td>7.70 ± 2.67(^k)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.90 ± 0.29</td>
<td>0.85 ± 0.49</td>
<td>0.98 ± 0.91</td>
<td>0.28 ± 0.16</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>2.18 ± 0.27</td>
<td>2.28 ± 0.30</td>
<td>1.85 ± 0.21</td>
<td>1.83 ± 0.43</td>
</tr>
<tr>
<td>Platelet (%)</td>
<td>666.75 ± 121.01</td>
<td>628.75 ± 108.75</td>
<td>404.75 ± 163.73</td>
<td>568.75 ± 65.05</td>
</tr>
<tr>
<td>PCT (%)</td>
<td>0.51 ± 0.06</td>
<td>0.49 ± 0.07</td>
<td>0.31 ± 0.12</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>PDW</td>
<td>15.63 ± 0.10</td>
<td>15.58 ± 0.18</td>
<td>15.73 ± 0.13</td>
<td>15.68 ± 0.08</td>
</tr>
<tr>
<td>MPV (Fl)</td>
<td>8.08 ± 0.29</td>
<td>7.88 ± 0.42</td>
<td>7.65 ± 0.17</td>
<td>7.98 ± 0.17</td>
</tr>
</tbody>
</table>

\(^a\)significant difference (P = .045) when compared with adult male rats
\(^b\)significant difference (P = .049) when compared with mid age male rats
\(^c\)significant difference (P = .02) when compared with mid age female rats
\(^d\)significant difference (P = .01) when compared with adult male rats
\(^e\)significant difference (P = .03) when compared with mid age male rats
\(^f\)significant difference (P = .03) when compared with mid age female rats
\(^g\)significant difference (P = .02) when compared with adult male rats
\(^h\)significant difference (P = .01) when compared with adult male rats
\(^i\)significant difference (P = .001) when compared with adult male rats
\(^j\)significant difference (P = .006) when compared with adult male rats
\(^k\)significant difference (P = .006) when compared with adult male rats
\(^l\)significant difference (P = .009) when compared with adult female rats
\(^m\)significant difference (P = .01) when compared with adult female rats

This study showed an increase in ulcer score in the adult male (0.80 ± 0.80) and mid age male rats (1.80 ± 0.92) colon though higher in mid age male rats (1.80 ± 0.92) while adult female, mid age female showed absence of ulcerations. Adult male and mid age male rats experienced loose stool but stool was normal for adult and mid age female rats. Bábičková et al. [23] also observed...
in their study that male mice had worse stool when compared with female adult mice. This effect may also be due to the protective effect of the estrogen and progesterone against the inflammatory [30].

In Figs. 2 - 5, all the sample rats shown no significant difference in malondialdehyde (MDA) concentration, catalase and superoxide dismutase (SOD) activities though reduced glutathione (GSH) concentration in mid age male rats increased significantly when compared with adult male rats (P< 0.05). This result is in agreement with the work of [18] who observed significant increase in glutathione (GSH) concentration fourteen days post colitis induction in colitis group when compared with control. There was an unclear pattern why higher increase in GSH was observed only in mid age male rats. Anti-oxidant enzymes such as the GSH, SOD and CAT have been reported to increase at the initial stage in the presence of inflammatory activities [27]. This is to protect the cell and prevent lipid peroxidation but in the situation of severe inflammatory activities or worsening of the disease condition, there is decrease in the levels of these enzymes which is indicative of oxidative stress [27].

Fig. 7. Photomicrograph of a colonic section stained by H&E showing histological changes in different groups: (A) Adult male rats showing a poorly preserved surface epithelial layer of the mucosal layer (white arrow), The tubular glands (crypt) and the lamina propria are moderately infiltrated by inflammatory cells (slender arrow) while the submucosal layer show severe infiltration of inflammatory cells (black arrow) and mild hemorrhage (B) Mid age male rats showing moderately preserved surface epithelia layer of the mucosal layer, The tubular glands (crypt) and the lamina propria are severely to chronically infiltrated by inflammatory cells (white arrow). The submucosal layer shows mild inflammation. (C) Adult female rats showing normal surface epithelia layer of the mucosal layer (white arrow). The tubular glands (crypt) and the propria are mildly infiltrated by inflammatory cells (slender arrow). The submucosal layer appear normal (black arrow) (D) Mid age female rats showing moderately preserved surface epithelial layer of the mucosal layer (white arrow), The tubular glands (crypt) and the propria are moderately infiltrated by inflammatory cells (slender arrow). The submucosal layer shows moderate infiltration of inflammatory cells (black arrow)
There was no effect of age and sex on the myeloperoxidase (MPO) activities in this study, there was no significant difference in MPO activities across the groups. Findings from [28] indicated an increase in MPO activities among male mice compared to the female mice while in the study by Babiekova et al. [23] there was no difference in the MPO activities among male and female mice. The difference in our finding and that of Wagnerova et al. [28] may be as a results of difference in number of days in post colitis induction study.

The effect of age and sex on the hematological parameters has varying results in this study. The elevated level of red blood cells (RBC) was observed the younger female rat compared to the older while it was reduced in the younger male rats compared to the older rats. Decrease in the red blood parameters has been linked to anaemia caused by low grade inflammation [29]. The reduction in younger rat compared to older adult is poorly understood but this may be due to higher metabolism rate of the younger rats [30]. There was no effect on PDW, PCT, Basophils, Eosinophils, platelet, MPV, MCV and WBC in this study. The HGB, MCH and Lymphocytes levels in the younger female rats were increased while Neutrophils and monocyte levels were decreased in the younger female rats only. It has been reported that the increase or decrease in the granulocyte cells indicate the increase or decrease of the inflammatory activities, [29]. From these results, it can be inferred that there was decrease of inflammatory activities in younger female rats as compared to other groups. This may be to the protective effect of estrogen [30].

The histomorphological study revealed poorly preserved surface epithelia layer and inflammatory cells infiltration in adult male rat, though mid age male section showed moderately preserved surface epithelia layer of the mucosal layer. The female rats section showed normal surface epithelia layer with mild inflammatory cells infiltration. From histomorphological study female rats were better healed than the male rats.

5. CONCLUSION

It can be concluded that age and sex influence the healing of ulcerative colitis. The effect of age and sex in the healing of the colitis is poorly understood. In terms of age, mid age rats (male and female) tends to heal faster than adult rats (male and female) while in terms of sex, female rats tends to heal faster than male rats.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the Ethical committee, Faculty of Basic Medical Science, Ladoke Akintola university of Technology, Ogbomoso, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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