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### **Administration of olive oil optimizes acetic acid induced colitis in CD1 mice**

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## Administration of olive oil optimizes acetic acid induced colitis in CD1 mice

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### ABSTRACT

Colitis is a chronic disease that affects individuals at different ages and affects the quality of their life. So far, there are three different experimental models for colitis. However, animals in these models die quickly due to the associate's toxicity. The aim of this study is to optimize acetic acid induced acute colitis mouse model with olive oil co-administration. Male albino CD1 mice (n= 91) were randomly divided into 13 groups (n= 7/group). To induce colitis, mice were administered with different volumes (100 or 200  $\mu$ l) and concentrations (1%, 2% and 4%) of acetic acid by intrarectal (IR) injection either for three consecutive days or every other day for a total of 6 days. Olive oil (1  $\mu$ l) was co-injected with acetic acid. One day later, mice were sacrificed and colon tissues were fixed in 10% formalin for histological examinations. The induction of colitis by acetic acid was dependent on both the volume and the concentration of acetic acid. Interestingly, co-administration of olive oil with different volumes and concentrations of acetic acid, although did not alter the severity of inflammation, it increased the animal survival rate and decreased the associated toxicity. Injection of co-administration of olive oil with acetic acid is a new modification to enhance the application of this model.

**Keywords:** Acetic acid, Colitis, colon, model

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### INTRODUCTION

Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis (UC) have a high incidence in industrialized nations and severely affect the life quality of patients (Amosy and Koma 2013). These diseases are defined clinically through inflammatory disorder in gastrointestinal tract accompanied with diarrhea, abdominal pains, weight loss, nausea and pathological changes such as a loss of mucosal integrity and inflammatory cell infiltration (Xavier et al., 2007). In addition, the presence of white blood cells. at the intestinal mucosal tissue is characteristic feature of this disease (Wang et al., 2005). Colitis affects the distal colon portion and induces non-transmural inflammation, massive necrosis of mucosal and sub-mucosal layers, neutrophil infiltration of the mucosa and sub-mucosal ulceration (Randhawa et al., 2014).

Induction of colitis might be as a result of the exposure to different inflammatory agents and

due to production of free radicals (Hanauer et al., 1996; Hanauer et al., 2006). Additionally, free radicals reduce antioxidant agents and thus increase the severity of the disease (Radko et al., 2007). Due to the activation of W. B. Cs and free radical production in the intestinal mucosal tissues, lipid peroxidation is elevated and may causes an inflammation (Bhattacharyya et al., 2014), resulting in increasing the permeability of blood vessels increased, the entrance of neutrophils to the site of inflammation and finally caused an expansion of the inflammatory area in the intestinal mucosal tissue (Cekici et al., 2014).

Furthermore, the release of inflammatory mediators and certain enzymes cause intestinal damage, injury, bleeding and diarrhea (Gasche et al., 2004). To treat colitis, the conventional therapeutic agents, for instance, the anti-inflammatory agents such as mesalazine, corticosteroids and immunosuppressive agents which act as non-specific drugs have been used

to reduce inflammation and the immune responses (John et al., 2011).

So far there are three identified models used to induce experimental colitis in rats and mice (Antonioni et al., 2016). These models include DSS, oxalone and acetic acid (MacPherson et al., 1978; Boirivant et al., 1998; Kawada et al., 2007). Although acetic acid-induced colitis is a good model to study the efficacy of compounds with potential anti-colitis (Cetinkaya et al., 2005; Tahan et al., 2011), induction of this model associates with severe injury, which leads to mortality in less than a week (Elson et al., 1995), limiting its application since it does not mimic the clinical setting in human. Therefore, optimizing this model to be more specific may help to test new effective treatments. With regard to the third model, there are a large number of reports that describe compounds that can ameliorate acetic acid-induced colitis (Choudhary et al., 2001, Karawya and Metwally 2016). Several compounds such as N-acetyl cysteine, trimetazidine, vitamin E, and melatonin were evaluated in acetic acid induced colitis model to decrease the reactive oxidative species (Wang Q et al., 2013; Uraz S et al., 2013).

The present study was conducted to induce a clinically relevant UC model by intrarectal (IR) administration of olive oil in combination with acetic acid doing induction of colitis. The results showed that injection of a 100  $\mu$ L of 4% acetic acid in combination with olive oil every other day for 6 days induced well characteristic colitis in mice but with less toxicity and better survival.

## MATERIALS AND METHODS

### Mice

Male Albino mice ( $n = 91$ ) weighed  $25 \pm 2$  g were acclimatized for one week before the study. Mice were purchased from housed in the Animal facility, Faculty of Science, Tanta University, Egypt. Animals were kept and maintained under a 12 h light/ 12 h dark cycle at a constant temperature ( $22 \pm 2^\circ\text{C}$ ) with a relative humidity of  $55 \pm 5\%$ . The experimental protocol was approved by the institutional Ethics Committee of the use of animals in research of Faculty of Science, Tanta University, Egypt .

### Reagents and chemicals

Acetic acid was purchased from Sigma Chemical Co., (St. Louis, Mo., USA) and reconstituted according to the manufacture description, and then diluted with saline to prepare 1, 2 and 4%. Olive oil (50  $\mu$ L) was obtained from Sigma Chem. Co., (St. Louis, Mo., USA), 50  $\mu$ L was mixed with 50 ml of acetic acid (1, 2 and 4% v/v in 0.9% saline).

### Induction of ulcerative colitis

Mice were administered with IR injection of 100  $\mu$ L or 200  $\mu$ L of 1, 2 and 4% acetic acid in combination with 1  $\mu$ L of olive oil. Immediately, after administration, the mice were hold horizontally for 2 min to prevent fluid leakage. Control animals were undergoing similar procedure using equal volume of olive oil instead of acetic acid as shown in Figures 1 and 3. As shown in Diagram 1, 13 groups were divided as follow: Gp1 to Gp7 were treated every other day for 6 days with olive oil (Gp1) or with different volumes and different concentrations of acetic acid in combination with 1  $\mu$ L of olive oil (Gp2 to Gp7). While the Gp8 to Gp13 were treated similarly to Gp2 to Gp7 but for three consecutive days. After 7 days of the 1st IR treatment, mice were fasted overnight and then anaesthetized by an intraperitoneal (i.p.) injection before sacrificing. The colon was excised extended proximally 2cm above anus opening (Morris et al.,1989).



**Figure 1.** Photomicrograph showed normal appearance of mouse anal opening dilated by inserting the retractors polyurethane cannula (external diameter 2 mm) was inserted into 3 cm proximal to the anus and instilled with an acetic acid solution.

## Histological preparation

Colon tissues from all the experimental groups were excised and cleaned by 0.9% saline and immediately fixed in 10% buffered formalin. For histological preparations, the colon tissues were washed until complete removal of formalin with running water. Tissues were then hydrated using different ascending concentrations of ethyl alcohol (50, 60, 70, 80, 90 and 100%) and then embedded by using 1:1 xylene: wax and 100% wax. Thin sections (5  $\mu$ m) were cut from the different blocks and processed according to the regular protocol for staining by eosin and hematoxylin (Bancroft et al., 1994). Finally, the stained sections were mounted on glass slides for histological examination by light microscopy and photographed.

## RESULTS

### Clinical symptoms

Injection with different volumes and concentration of acetic acid in the presence of olive oil led to some clinical symptoms which indicated to the presence of colitis such as bleeding as shown in Figure 2a, weight loss (data not shown) and diarrhea (Figures 3 a,b). As shown in Figure 4a, administration of acetic acid and olive oil led to induction of ulcerative colitis.

### Histopathological changes of colitis induced by acetic acid

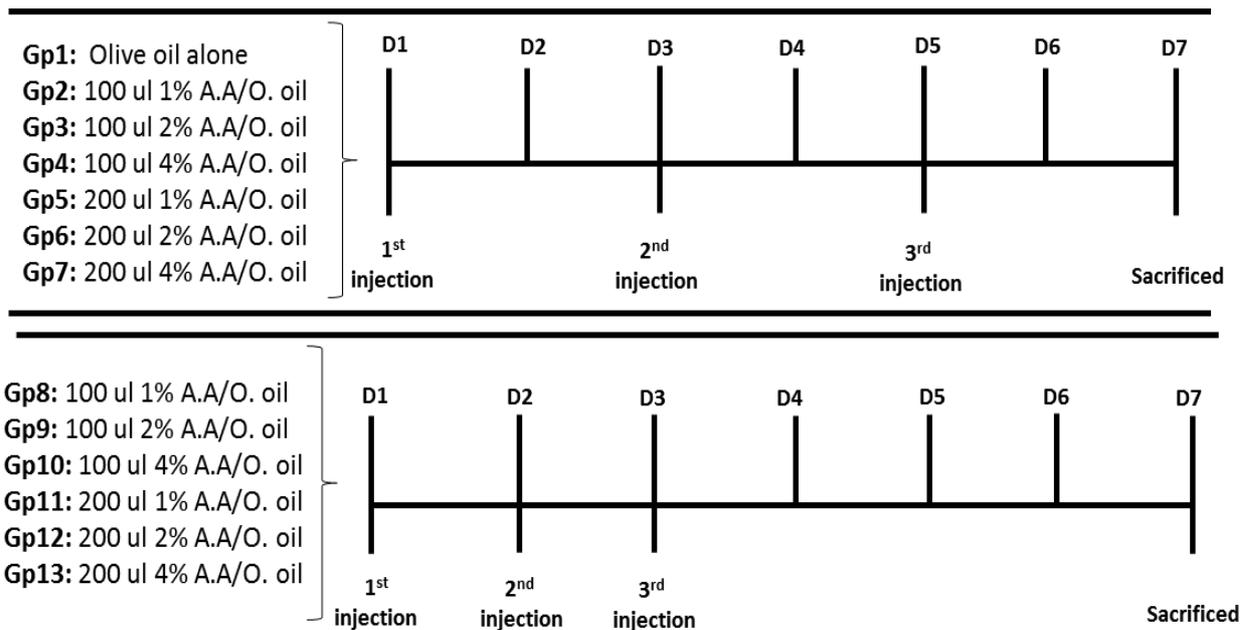
To investigate the pathological changes in the colon tissues in the different groups (Gp1 to Gp13), the microscopic examination was conducted. The results showed H and E-stained colon sections of control group (Gp1) revealed normal histological structures of the different layers of the colon. The colon mucosa showed normal intestinal crypts lined by epithelial columnar cells and goblet cells. The lamina propria contained few mucosal mast cells full of cytoplasmic granules (Figure 4a). Investigation of colon sections of groups of mice treated with different volumes and concentrations of acetic acid showed a variety of histological abnormalities in colon structure, which clearly indicated to the induction of colitis of these groups.

The present study clearly showed that intrarectal (IR) administration of 100  $\mu$ L acetic acid combined with 1  $\mu$ L of olive oil into male albino mouse induced colitis in groups as measured by the grade of ulcers as compared to control (Figures 4, b-f and 5 a-f). Of note, the IR administration of 200  $\mu$ L acetic acid of different concentrations (1,2 and 4%) led to high mortality as compared to 100  $\mu$ L (data not shown).

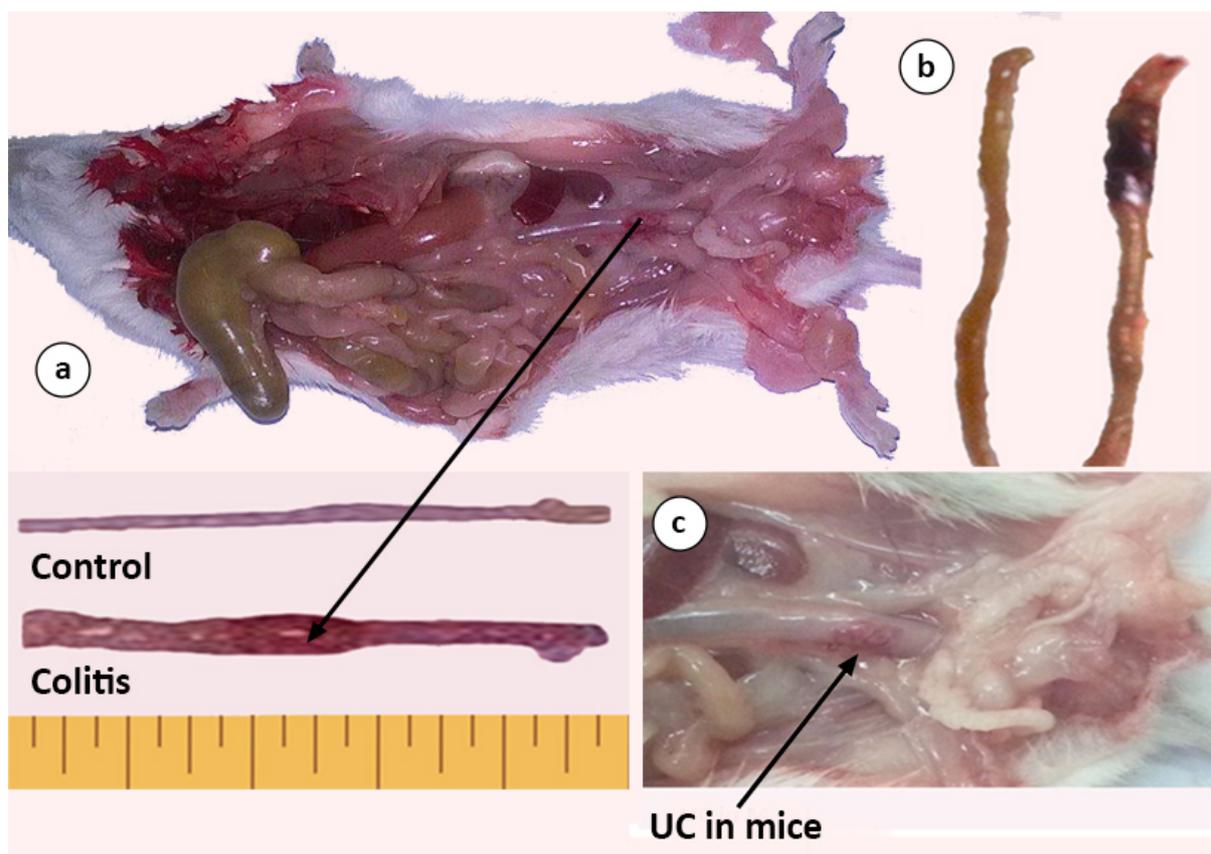
Abnormal structures in all layers of colon were found in particular, a marked epithelial cell damage was found represented by the form of epithelial separation, cellular loss, congestion of blood vessels, crypt hyperplasia and focal depletion of goblet cells. Additionally, severe mononuclear cellular infiltration in lamina propria in the muscularis mucosa and the submucosa were observed (Figure 4b-g). Interestingly, these abnormalities in colon were increased with the increase of acetic acid concentrations. As shown in Figures 5 a-f, and 2c, the changes in the colon in mice treated with different concentrations of acetic acid every other day for 6 days with 100  $\mu$ L of 1% acetic acid revealed mild a trophy (arrows) in colon mucosa with mild inflammations. The results showed that colon sections in treated mice with 100, 200  $\mu$ L acetic acid at concentrations of 1% 2% and 4% induced a strong damage in colon with marked inflammation (stars) and colitis, respectively (Figure 5b-f). Interestingly, treatment with different concentrations of acetic acid induced diarrhea which increased with the time after the treatment as shown in Figures 1 and 3b. associated with bleeding as seen in Figure

## DISCUSSION

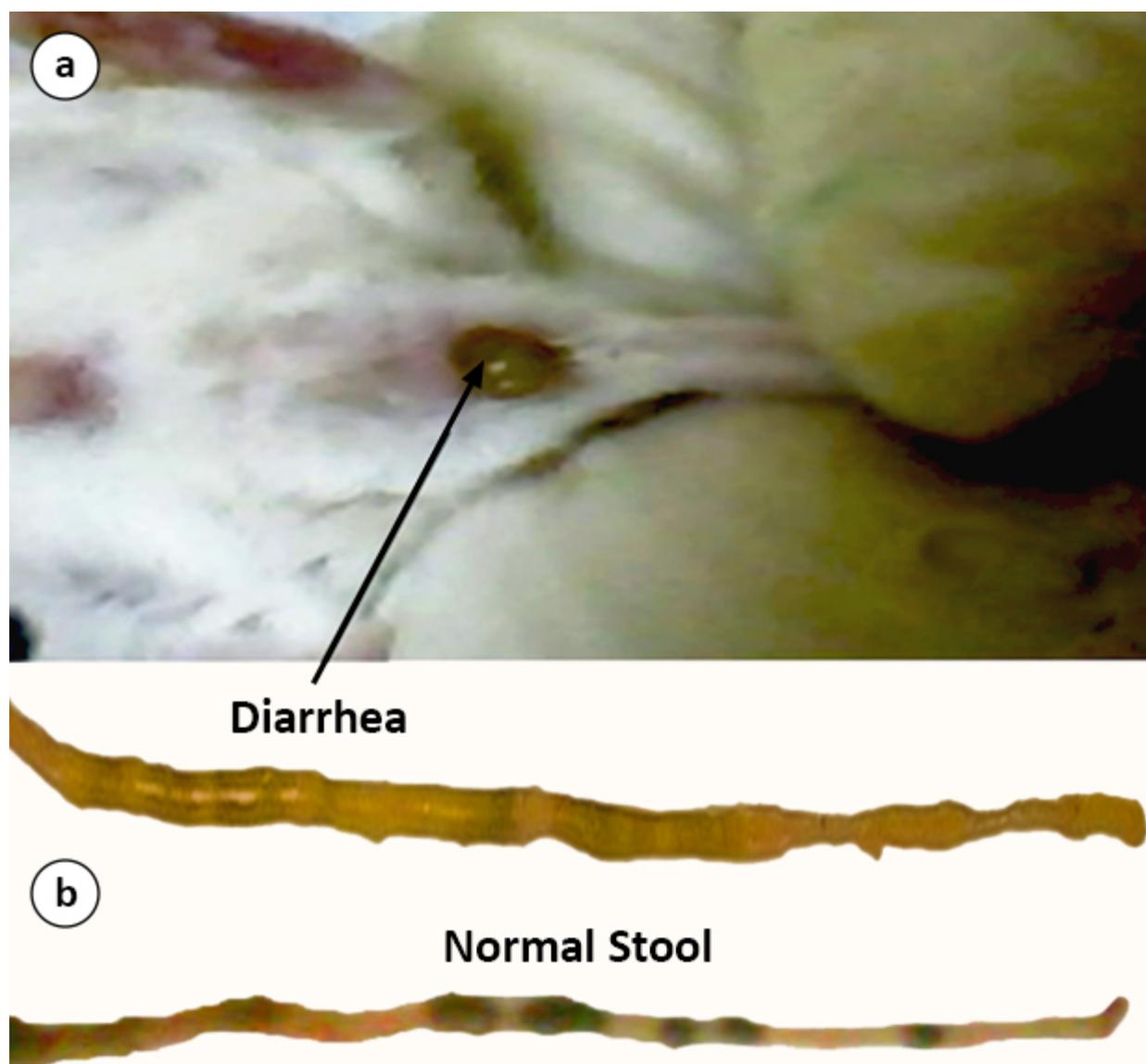
Colitis is IBDs are chronic and relapsing inflammatory disorders of the gastrointestinal tract combined with diarrhea, weight loss and nausea and by different pathological features such as a loss of mucosal integrity and inflammatory cell infiltration (Xavier et al., 2007). So far, there are three identified models used to induce experimental colitis in rat and mice (Bramhall 2015; Almeida et al., 2017). These models include DSS, oxalone and acetic acid (MacPherson et al., 1978; Boirivant et al., 1998; Kawada et al., 2007).



**Diagram 1.** Shows the experimental diagram the 13 groups under the study, A, A; acetic acid, O. Oil; olive oil.



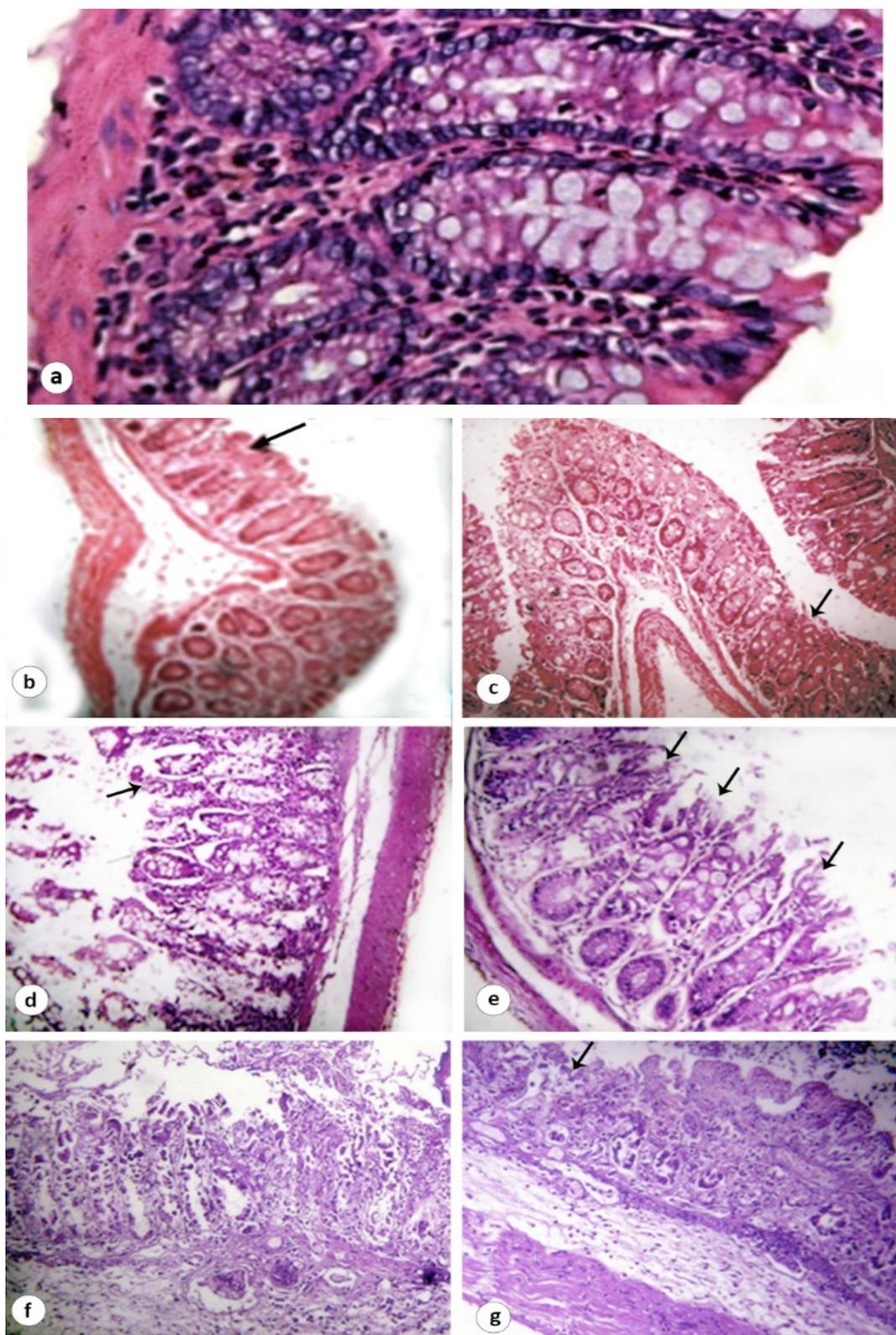
**Figure 2.** a) Photomicrograph showed that acetic acid-induced ulcerative colitis, b) Photomicrograph showed the colon inflammation associated with bleeding, clearly showed control group and colitis group. c) Photograph showed the induction of colitis in mice treated with acetic acid and olive oil.



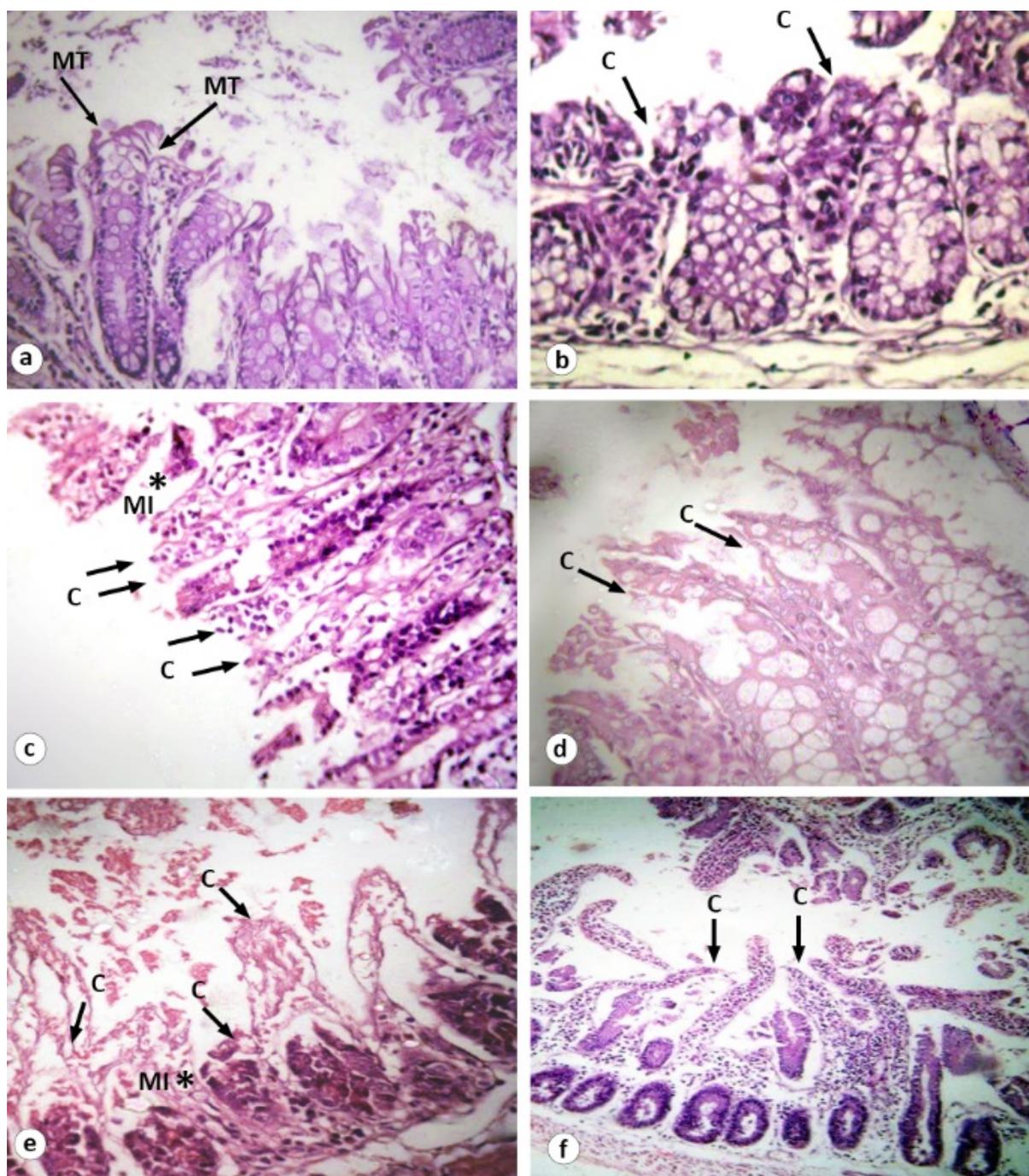
**Figure 3.** a) after acetic acid induction all the mice eventually had diarrhea increased over time, outside of the anus. b) Photomicrograph showed the stool consistency. a: normal appearance of stool in control colon & diarrhea in colitis colon.

Concerning the third model, there are a large number of reports that, described the possibility of several potential compounds can ameliorate acetic acid-induced colitis (Lean et al., 2015). Other studies showed also that, many drugs as well as diet could worsen or improve colonic functions and symptoms according to type and severity of colitis (Valdez et al., 2000). Several compounds such as N-acetyl cysteine, trimetazidine, vitamin E, and melatonin were tested by using acetic acid

colitis model to decrease the reactive oxidative species (Kurutas et al., 2005). On the other hand, other colitis models are accompanied by side effects. some critical parameters and troubleshooting as DSS-induced colitis model depend on numerous key factors, including DSS source, molecular weight, concentration, duration, mouse strain, source, age, gender and body weight as well as environmental factors including the hygienic condition of the vivarium (Nell et al., 2010).



**Figure 4.** a) Hematoxylin and Eosin sections showed the histological structure of colon mouse in control group. (Gp1) injected with olive oil every other day for 6 days showed normal histological structure. (100X). (b-e) Hematoxylin and Eosin sections showed the histopathological changes in colon of mice treated with different volumes and concentration of acetic acid with tiny volume of olive oil for 3 successive days. b: Mice treated with 100  $\mu$ L acetic acid revealed mild atrophy (arrows) in colon mucosa with mild inflammations. c: Colon sections in treated mice with 100  $\mu$ L of 2% acetic acid revealed moderate colitis (arrows). d: Colon sections of treated mice with 100  $\mu$ L of 4% acetic acid revealed moderate colitis (arrows). e-g: Colon sections in treated mice with 200  $\mu$ L of 1%, 2%, 4% acetic acid, respectively, revealed strong damage in colon with marked inflammation (stars) and colitis (arrows). b - f (200X), f (400X).



**Figure 5.** Hematoxylin and Eosin sections showed the histopathological changes in the colon in mice treated with different concentration of acetic acid every other day for 6 days. **a:** Mice treated with 100  $\mu$ L of 1% acetic acid revealed mild a trophy (arrows) in colon mucosa with mild inflammations. **b-f:** Colon sections in treated mice with 100 or 200  $\mu$ L of 1% 2% and 4% of acetic acid, respectively, revealed strong damage in colon with marked inflammation (stars) and colitis (arrows). **a – f** (200X), **d** (400X).

The aim of present study was to establish a clinically relevant ulcerative colitis model that induced by different concentrations of acetic acid. In this study, the results showed that the IR administration with acetic acid in combination with olive oil into male albino mouse induced colitis. This indicating that acetic acid damage was effective and had the

ability to increase the abnormalities in the intestinal mucosal tissues, mucosal injury, degeneration in epithelial cells of mucosa, depletion in goblet cells. Off note the presence of olive oil as a component of the treatment with acetic acid reduced the toxic effect of acetic acid upon the injection because of olive oil prevented the development of aberrant

crypt foci and colon carcinomas, eating olive oil can improve the efficiency of intestines. Olive oil also encourages intestines to absorb more of the vitamins and minerals from the foods (Tsimidou et al., 2015). Additionally, IR administration with acetic acid into male albino mouse increased the leucocytic infiltrations, abnormal changes in the intestinal crypt and a loss of mucosal integrity. Microscopic examination of colon tissues of mice is IR administered with different concentrations with acetic acid in combination with tiny volume of olive oil showed an increase in the number of neutrophil and infiltration of inflammatory cells. Our finding was in agreement with previous studies which showed that acetic acid induced colon inflammation (Hartmann et al., 2012).

In conclusion having olive oil as a component of the injection with different volumes and concentrations of acetic acid induced colitis with less severity and low mortality rate. The results postulated that the combinatorial treatment with a 100 µL of 4% acetic acid and a tiny volume of olive oil was the optimal to induced colitis.

## ABBREVIATIONS

IBD; Inflammatory Bowel Diseases, CD; Crohn's Disease UC; Ulcerative Colitis.

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