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Research Paper: Ammonium chloride-induced ulcerative colitis in rat: A novel reproducible experimental model

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ABSTRACT

Objective: Although chemicals including trinitrobenzenesulfonic acid (TNBS) and dextran sodium sulfate (DSS) are widely used to induce ulcerative colitis (UC) in laboratory animals, they are expensive and have side effects. This study aimed to introduce a novel model of chronic UC using ammonium chloride as an inexpensive material.

Materials and Methods: In this *in-vivo* study, 21 adult male Sprague Dawley rats were divided into three equal groups as follows: the first group (control) was received 0.5 cc of distilled water and the second and third groups were received 0.5 cc of ammonium chloride at concentrations of 2 mol/L or 4 mol/L through rectal enemas for 14 consecutive days (once daily). The procedure was stopped for two weeks, and then started and continued till rectal bleeding was observed. At the end, animals were sacrificed and colon, liver, and kidney tissues were examined histopathologically.

Results: Although gross observation of colons in the control group showed a normal structure without histopathological changes, rectal enemas with 2 mol/L ammonium chloride caused hemorrhagic areas as well as mild edema of the sub mucosal layer and inflammatory cell infiltration. Besides, rectal enemas with 4 mol/L ammonium chloride caused an extensive ulceration/necrosis, severe inflammation and edema, moderate fibrinous exudate, and mild atrophy of intestinal glands. The liver and kidney tissues were normal in all groups.

Conclusion: Based on the findings, ammonium chloride can be used as an inexpensive alternative for inducing a chronic model of UC in rat. Current model also fulfills the histopathological criteria of UC.

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1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory disorder of the colorectum with low incidence, recurrent symptoms, significant morbidity, and rare fatality. It causes continuous mucosal inflammation and ulcers extending from the rectum to the more proximal colon. Despite extensive research on microbiological, immunological, biochemical, and epidemiological aspects of UC, the exact etiology of the disease and its pathogenesis remain obscure. However, numerous environmental, microbial and genetic factors have been contributed to the pathogenesis of UC (1-3).

A wide variety of animals have been used in biomedical researches to simulate acute and chronic UC. Although the development of UC in animal models does not address all the pathophysiological aspects of the disease observed in humans, animal models are still crucial for analyzing disease-causing mechanisms and designing new therapeutic approaches. Besides, it would be difficult to conduct such diseases in humans. Generally, animal models of UC can be divided into four categories including spontaneous models, genetically manipulated models, cell transfer models, and chemically-induced models (4-7).

Chemically-induced UC models are able to mimic the morphological, histopathological and symptomatical features of the disease and also suitable for dissecting the pathogenic/regulatory components during an acute, recovery and chronic phases of colitis. However, these models need to optimize the concentration of chemical agents based on animal facilities (8, 9). Over time, various chemicals such as trinitrobenzenesulfonic acid (TNBS), dextran sodium sulfate (DSS), acetic acid, oxazolone, non-steroidal anti-inflammatory drugs (NSAIDs), carrageenan, and peptidoglycan-polysaccharide (PGPS) have been used to induce UC (4-6). Low cost and the ease of administration are the main advantages of chemical agents rather than other methods; however, some of these chemicals are expensive for long-term use and can cause a range of side effects. Besides, UC induced by some chemicals shows acute features that self-limiting without any treatment (4-6, 10, 11) whereas UC in human is a long-term (chronic) inflammatory disease with periods of remission and relapse (3).

Currently, the oral administration of sulfated polysaccharides such as DSS that mixed in drinking water as well as the chemical irritation through rectal instillation of sensitization to TNBS has been frequently used for inducing UC in laboratory animals. However, the disease in animals just manifests following the vast consumption of these expensive chemicals for a long time (12-14).

Ammonium chloride (NH_4CL), also known as Sal ammonia is a colorless salty product derived from ammonia and hydrogen chloride reaction. It is readily available and inexpensive. The crystalline substance of ammonium chloride is highly soluble in water, which result in a slightly acidic solution. Ammonium chloride is a non-corrosive, non-destructive and non-toxic chemical. It is permitted as a pharmacologically active substance in veterinary medicinal products as well as a buffer solution to control pH levels in a wide variety of chemical and medical applications (15-17). Based on previous findings, this study aimed to introduce an inexpensive and reproducible model of chronic UC using the ammonium chloride rectal enema.

2. Material and Methods

Animals and Ethics

Twenty-one adult male Sprague-Dawley rats (200-230 g) were purchased from Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. The animals were housed in cages and adaptively acclimatized for 7 days at controlled temperature (22±2°C), lighting (12 h light/dark cycles), and a 60% humidity. All study procedures were approved by the local Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (Approval ID: IR.SUMS.REC.1397.968). Animals were handled in accordance with the established guidelines.

Induction of Ulcerative Colitis

Rats were randomly assigned into three equal groups (7 per each) as follows: the first group (control) was received rectal enemas with 0.5 cc of distilled water, and the second and third groups were received rectal enemas with 0.5 cc of ammonium chloride at concentrations of 2 mol/L or 4 mol/L, respectively. Briefly, all rats were anesthetized with CO2 inhalation and then, a lavage needle was inserted into the anus. The tip of the needle was advanced to 8 cm proximal to the anus verge. The ammonium chloride solution or distilled water was injected into the colon and rats were kept in a head-down position for 1 min to avoid leakage. Then, they were returned to their cages with free access to standard rat chow and water. In each group, rectal enema was repeated once daily for 14

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consecutive days and then, the procedure was stopped for a period of two weeks. Rectal enemas were started again as described above, and continued till rectal bleeding was observed. With the onset of bleeding in the first rat, all rats were sacrificed and the body tissues were harvested for histopathological examinations.

Histopathological Assessments

At first, the colon, liver, and kidney tissues were examined macroscopically and then, they were fixed by immersion in 10% neutral-buffered formalin. To prepare tissue blocks, the samples were subjected to routine histological processes using an automated tissue processing machine (DID SABZ Co., Iran) and were then embedded in paraffin. Consequently, the microtome (DID SABZ Co., Iran) was set at 5 µm thickness and the sections were prepared. Tissue slides were stained with the conventional hematoxylin and eosin method (Merck, Germany) and were examined under a light microscope (Nikon Eclipse Ni, Japan) by a pathologist blinded to the study.

3. Results

Rectal bleeding was first detected in rats treated with 4 mol/L of ammonium chloride at the 9th day of the secondary enema. In control group, the gross observation of the colon showed a normal structure without histopathological changes. However, numerous hemorrhagic areas were observed in the colon of rats treated with 2 mol/L of ammonium chloride. Histopathological assessments in this group also showed mild edema of the sub mucosal layer of the colon and inflammatory cell infiltration without evidence of necrosis, fibrinous exudate, or atrophy of intestinal gland. The gross observation of colons in rats treated with 4 mol/L of ammonium chloride showed extensive hemorrhagic and necrotic areas. Besides, a series of histopathological changes including extensive ulceration/necrosis, severe inflammation and edema of the sub mucosal layer extended into the tunica serosa, moderate fibrinous exudate, and mild atrophy of intestinal glands were observed in this group. Macroscopic and microscopic features of colons in experimental groups are presented in Figures 1 and 2. It should be noted that both macroscopic and microscopic assessments of kidney and liver tissues showed normal structures in all groups (Figure 3).

Table 1. Histopathological findings of colon in experimental groups. The results were expressed as the median score

Histopathological findings	Experimental groups		
	Control	Ammonium chloride-treated groups	
		2 mol/L	4 mol/L
Ulceration/Necrosis	-	-	+++
Edema	-	+	++
Inflammatory cell infiltration	-	+	++
Fibrinous exudate	-	-	++
Atrophy of intestinal gland	-	-	+

- No evidence; + Mild; ++ Moderate; +++ Severe

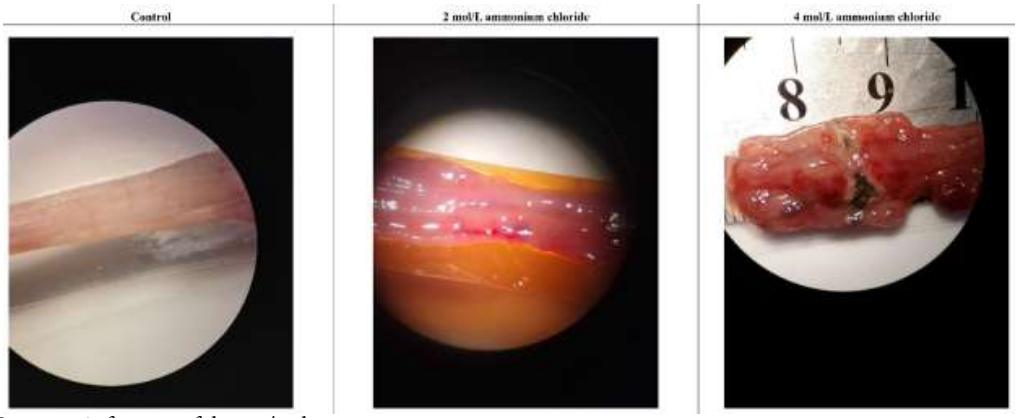

Figure 1. Macroscopic features of the rats' colons
The gross observation of colons with stereo microscope exhibited a normal anatomy in control group; however, colons in those treated with 2 mol/L ammonium chloride or 4 mol/L ammonium chloride were associated with hemorrhagic areas or extensive necrotic and hemorrhage areas, respectively.

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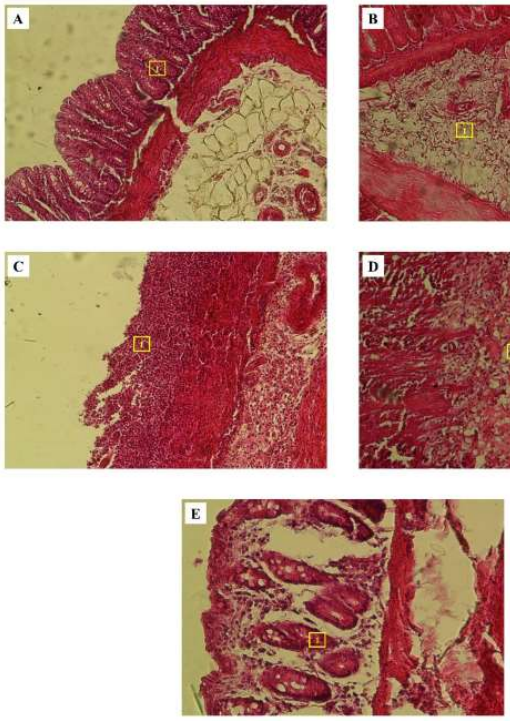

Figure 2. Microscopic features of the rats' colons.
A) Normal structure of colon (1) in control group (H & E staining; ×100). B) Mild edema of the sub mucosal layer (1) and mild inflammatory cell infiltration (1) without ulceration/necrosis, fibrinous exudate, and atrophy of intestinal glands in rats treated with 2 mol/L ammonium chloride (H & E staining; ×100). C) Severe ulceration/necrosis of the epithelium (1) in rats treated with 4 mol/L ammonium chloride (H & E staining; ×100). D) Severe edema of the sub mucosal layer (1), severe inflammatory cell infiltration (1), and moderate fibrinous exudate (2) in rats treated with 4 mol/L ammonium chloride (H & E staining; ×100). E) Mild atrophy of intestinal glands in rats treated with 4 mol/L ammonium chloride (H & E staining; ×100).

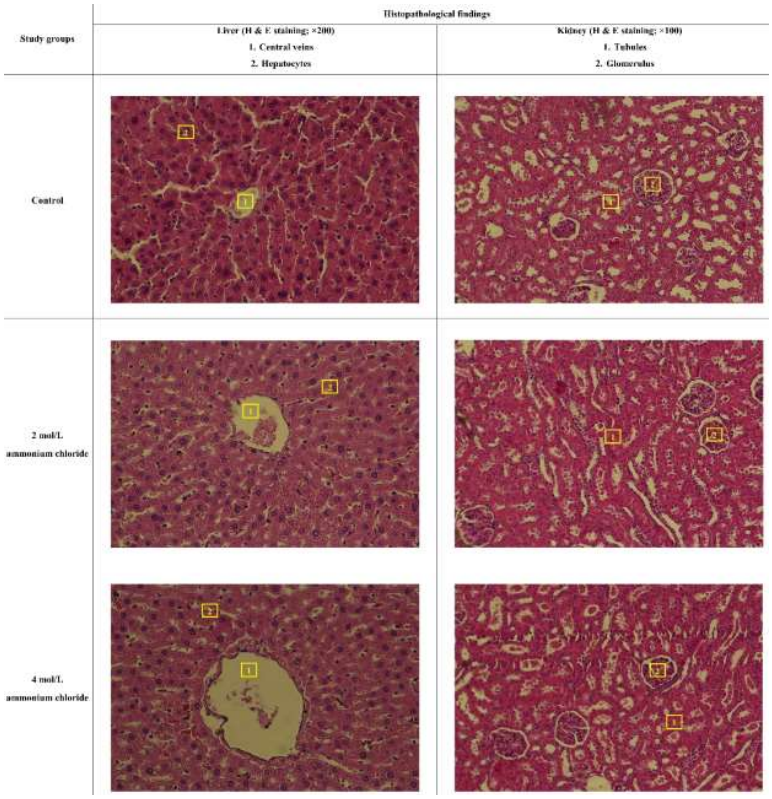

Figure 3. Normal structures of liver and kidney tissues in experimental groups

4. Discussion

The current study was designed to establish an inexpensive model of experimental UC in rat using rectal enemas with different concentrations of ammonium chloride. Our findings revealed an extensive hemorrhagic and necrotic areas through the gross observation of the colon and a series of histopathological changes including extensive ulcer, necrosis of the epithelium, heavy inflammation and edema of the sub mucosal layer extended into the tunica serosa, the fibrinous exudate, and the atrophy of intestinal glands in rats treated with 4 mol/L of ammonium chloride solution.

UC usually presents with bloody diarrhea and is diagnosed by colonoscopy and histological findings (3). It is impossible to assess rectal bleeding in all animals at any time of the day or night. Besides, rats may have minor bleeding to be observe by investigators. Hence, bloody diarrhea is considered as the characteristic symptom of UC in our animals, which was further confirmed by histopathological assessments. Generally, the mucosal layer that preserves the digestive lumen is damaged by several mechanisms through the development of UC (18). Key histological features of UC is diffuse superficial mucosal infiltration, erythema of inflamed mucosa, areas of bleeding, and ulceration (19), which are compatible with the findings of this study.

Although previous studies were reported acute gastric mucosal lesions and colon mucosal cell damage following an oral administration of ammonia in mice and rat, the ammonium chloride administration not only had no such effects but also exerted the protective activity on gastric mucosa (20-22). It should be noted that ammonium chloride can activate the mitochondrial urea cycle enzymes, including glutamine, without any increase in the plasma levels of hepatic or renal enzymes (23). Glutamine can stimulate the production of glutamine reductase which is involved in the removal of reactive oxygen species (ROS) in digestive tract (24). The stimulatory effect of ammonium chloride on gluconeogenesis in the hepatic cells from the source of glutamine was also reported (25). Since this study used enemas as a method of drug administration instead of oral route, the abovementioned side effects of oral administration of ammonium chloride were resolved. On the other hand, absolute increase in kidney weight (26), urolithiasis (27), chronic metabolic acidosis (28), and a mild stimulating effect on the airway secretory cells (23) were also reported following the ammonium chloride feeding in rodents. In contrast, the side effects on visceral organs were not observed in this study and kidney and liver tissues had normal structures. Therefore, the present model seems to be safe and reproducible. UC in human is a long-term (chronic) inflammatory disease in which abnormal reactions of the immune system cause inflammation and ulcers on the inner lining of large intestine. Besides, the clinical course of UC is characterized by alternating periods of remission and relapse; however, it is unpredictable (3, 29). Hence, the process of enema with ammonium chloride solution was stopped for two weeks and started again to induce a chronic model of ulcerative colitis, which is more compatible with that observed in human.

This study also has several limitations. First, we used rectal enema with ammonium chloride for the first time; hence, all injecting doses of ammonium chloride were selected based on the preliminary studies. Second, we just evaluated the colon, kidney and liver tissues histopathologically; therefore, the effects of ammonium chloride on other body tissues should be assessed in future studies. Finally, the exact mechanisms of ammonium chloride-induced UC and the type of role-played inflammatory cells in the site of injuries were also not determined.

Conclusion

Rectal enemas with ammonium chloride solution can induce UC in rats and this is an inexpensive, safe and reproducible method. Gross and histopathological observations did not show any adverse effects on kidney and liver tissues; however, our model of UC represented the major characteristics of chemical-induced colitis.

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Conflict of Interest

The authors declare no conflict of interest.

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