DOI: https://doi.org/10.31351/vol32iss2pp33-40

Evaluation of Anti-inflammatory Effects of Cinnamic Acid Against Dextran Sodium Sulfate-Induced Ulcerative Colitis in Male Mice

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Abstract

Anti-inflammatory effect of cinnamic acid on the dextran sodium sulfate (DSS) induce ulcerative colitis in mice. Inflammatory bowel disease (IBD) is a common chronic inflammatory disease of the gastrointestinal tract, including ulcerative colitis and Crohn's disease. ulcerative colitis (UC) disease is characterized by chronic, persistent, recurrent, and nonspecific intestinal ulcer and mucosal inflammation. In this study, the protective effects of cinnamic acid on the dextran sodium sulfate (DSS) induced ulcerative colitis in mice was investigated. Forty adult male mice were collected and randomly divided into five groups, group I received a suspension of distil water and poloxamer, group II received 3% DSS in drinking water for seven consecutive days. Two treatment groups received oral suspension of cinnamic acid 50 and 25 mg/kg respectively and 3% DSS in drinking water, for seven consecutive days and the final group received oral suspension of cinnamic acid 50mg/kg for 7 days without DSS in drinking water. After all the animals were euthanized on day eight. The samples were collected for analysis. DSS with cinnamic acid 50 mg/kg group revealed a significant ($p<0.05$) reduction in Tumor necrosis factor α, interleukin 6, nuclear factor-κB, cinnamic acid 25mg/kg revealed a significant ($p<0.05$) reduction in Tumor necrosis factor α and nuclear factor-κB but not significant ($p>0.05$) reduction in IL-6 when compared to the model group. Histopathological examination showed a significant reduction of inflammatory signs in all cinnamic acid treated groups when compared to the DSS model group.

The treatment with cinnamic acid significantly decreased the levels of DSS induce pro-inflammatory cytokines. This finding supports the idea that the use of this substance could be used as a potential therapy for patients with ulcerative colitis.

Keywords: Inflammatory bowel diseases, Ulcerative colitis, Dextran sodium sulfate (DSS), Pro-inflammatory cytokines, Cinnamic acid.

تقييم الآثار المضادة للالتهابات من حمض القرفة ضد كبريتات الصوديوم ديكستران الناجمة عن التهاب القولون التقرحي في الفئران الذكور

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الخلاصة

هو دراسة التأثيرالمضاد للالتهاب لحمض سينماميك على ديكستران كبريتات الصوديوم (DSS) التي تسبب التهاب القولون التقرحي في الفئران. مرض التهاب الأمعاء هو مرض التهابي مزمن شائع في الجهاز الهضمي، بما في ذلك التهاب القولون التقرحي ومرض كرون. يتميز مرض التهاب القولون التقرحي بقرحة معوية مزمنة ومستمرة ومتكررة وغير محددة والتهاب الغشاء المخاطي. في هذه الدراسة، تم التحقيق في الآثار الوقائية لحمض القرفة على كبريتات الصوديوم الدكستران الناجمة عن التهاب القولون التقرحي في الفئران. تم جمع أربعين فأرا من الذكور البالغين وتقسيمهم عشوائيا إلى خمس مجموعات، تلقت المجموعة الاولى معلق من الماء المستخلص، تلقت المجموعة الثانية ٣٪ من كبريتات الصوديوم الدكستران لمدة ٧ أيام متتالية، تلقت مجموعتان من مجموعات المعالجة معلقا فمويا لحمض القرفة ٥٠ و٢٥ ملغ / كغم على التوالي و٣ ٪ من كبريتات الصوديوم الدكستران لمدة ٧ أيام متتالية وتلقت المجموعة النهائية معلقا عن طريق الفم من حمض القرفة ٥٠ ملغم / كغم لمدة ٧ أيام دون كبريتات الصوديوم الدكستران في مياه الشرب. بعد أن تم القتل الرحيم لجميع الحيوانات في اليوم الثامن، تم جمع العينات للتحليل، نتج عن إعطاء حمض القرفة وكبريتات الصوديوم الدكستران انخفاض كبير (TNF α, IL-6, NF-κB)، ونتج عن إعطاء حمض القرفة ٢٥ ملغم/كغم انخفاض كبير في TNF α, NF-κBلكن لم يسبب انخفاض كبير بانترلوكين - IL-6. أظهر الفحص النسيجي المرضي انخفاضا كبيرا في العلامات الالتهابية في جميع المجموعات المعالجة بحمض القرفة عند مقارنتها بمجموعة نموذج DSS .

أدى العلاج بحمض القرفة الى انخفاض كبير في مستويات السيتوكينات الالتهابية المؤيدة المرتبطه بكبريتات الصوديوم الدكستران تدعم هذه النتيجة فكرة أن استخدام هذه المادة يمكن استخدامه كعلاج محتمل للمرضى الذين يعانون من التهاب القولون التقرحي.

الكلمات المفتاحية: امراض الأمعاء الالتهابية، التهاب القولون التقرحي، كبريتات الصوديوم الدكستران، السيتوكينات المؤيدة للالتهاب، حمض القرفة

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Received: 1/7 /2022
Accepted: 1/ 9/2022

Introduction

Inflammatory bowel disease, which includes Crohn's disease and ulcerative colitis, is a chronic condition that affects the gastro-intestinal tract (GI) tract. It can lead to various health problems, including cancer. The link between colorectal cancer and inflammatory bowel disease (IBD) has been identified as a significant factor that affects the quality of life in developing nations[1].

The damage to the large intestine's mucosal layer is a chronic and persistent condition known as ulcerative colitis. It can manifest in a continuous manner and cause superficial damage to the surrounding tissue. The main cause of this condition is the loss of the epithelial barrier's integrity. The lack of an immunological response and the recruitment of leukocytes are also known to trigger the development of IBD. The symptoms of ulcerative colitis include abdominal pain, frequent diarrhea, and a burning feeling in the belly. It can also lead to weight loss[2,3]. Other factors that can also contribute to the development of this condition include the presence of environmental factors and body's immunological responses. These factors can also decrease the body's antioxidant capacity[4].

High levels of these pro-inflammatory cytokines can also play a role in the development of this condition. They can also help determine the severity of the disease[4].

A water-soluble compound known as dextran sodium sulfate, which has a variable molecular weight ranging from 5 to 1400 KDa, is commonly used in animal models to induce colitis. It can be obtained by adding a dose of around 40 to 50 KDa to drinking water[5].

The exact mechanism of the DSS effect on the development of colitis is not known. However, it is believed that it can cause damage to the lining of the large intestine, which could allow the release of proinflammatory intestinal contents[5].

It is believed that the link between IBD and the immune system can be caused by a combination of factors. One of these is the interaction between the microorganisms and the immune system This interaction can trigger an inflammatory response and alter the function of the colonic system[4].

Anti-inflammatory drugs are commonly used in the treatment of ulcerative colitis (Amminosylates and glucocorticoids are also commonly used in moderate and mild cases). In addition to anti-inflammatory drugs, immune suppressants are also commonly used in the treatment of ulcerative colitis. These include azathioprine and cyclosporine A[6]. The use of immunosuppressive drugs and antibiotics for treating ulcerative colitis can also have side effects. This led to the risks of adverse effects upon long term treatment that limit their clinical use. As a result, they are often replaced with safer and more tolerable alternatives[4].

Due to the limited side effects of food derivatives, they have been considered as potential treatment agents for patients with IBD. Over the past decade, various food components have been studied as potential chemo preventive agents for UC[6].

A cinnamic acid compound is an aromatic fatty acid that has low toxicity and is commonly used in the design of anticancer drugs. It is composed of a phenyl ring and an acrylic acid group. It exhibits an active moiety that can be utilized in the development of drugs[7]. Cinnamic acid is a key chemical found in plants such as “Cinnamomum cassia (Chinese cinnamon) and Panax ginseng, fruits, whole grains, vegetables and, honey”. It can help protect them from harmful effects from ultraviolet radiation. It can also help in the growth and development of organisms[7]. “Cinnamon”, The general term for the flavor of cinnamon is, it has been used as a food ingredient for a long time due to its sweet and spicy taste. in addition, for being used as a flavor for chewing gum, cinnamon has also been known to improve the function of the liver's glycogen synthase and inhibit the growth of cancer cells. It can also help remove bad breath. In addition, it has been used to treat various conditions such as cancer and angiogenesis, and vascularization[8]. This substance also helps in the treatment of neurogenerative diseases. It can also help in the prevention of hypertensive and cardiovascular diseases[9].

Cinnamic acid has a cardioprotective effect in a rat model of ischemic myocardial injury. The protection was attributable to its anti-inflammatory properties by decreasing the proinflammatory cytokines including TNF-α, NF-κB and IL-6 [10, 11].

Materials and Methods

Chemicals and Kit

The chemical that were used in this work includes , Diethyl ether (ROMAN pure chemistry, UK) , Formaldehyde 37% (Sinopharm chemical reagent Co., Ltd/China) , dextran sodium sulfate (Med ChemExpress), Poloxamer188 from (Thermo fisher scientific) , Cinnamic Acid (SIGMA-ALDRICH/ China), Buffer solution (EuroClone S.p.A ,Italy), Distal water, all enzyme-linked immunosorbent assay (ELISA) kit utilized in the study were obtained from Bioassay Technology Laboratory, China and include mouse Interleukin 6(IL-6) ELISA Kit, mouse Tumor necrosis factor α (TNF- α) ELISA Kit, mouse Nuclear factor -kappa B ELISA Kit.

Animals

Forty adult male albino mice (aged 8 weeks) were included in this study, with weights ranging from 24-31g. Mice were kept in standard conditions of temperature, day/night cycle, with free access to the standard diet. Dextran sodium sulfate dissolved in the drinking water for seven days has been used to induce ulcerative colitis.

Experimental protocol

Forty mice were divided into five groups, each group composed of eight mice in each group, as the following:

1. Group I (Control group): Eight mice received a suspension of distil water and poloxamer (the suspending agent) by Oral gavage for 7 days.

2. Group II (Model group): Eight mice received a suspension of distil water and poloxamer (the suspending agent) by Oral gavage for 7 days. In addition to the administration of 3% DSS with drinking water during the seven days.

3. Group III (cinnamic acid-model group): Eight mice received a suspension of 25 mg/kg cinnamic acid by Oral gavage for 7 days. In addition to the administration of 3% DSS with drinking water during the seven days.

4. Group IV (cinnamic acid -model group): Eight mice received a suspension of 50 mg/kg cinnamic acid by Oral gavage for 7 days. In addition to the administration of 3% DSS with drinking water during the seven days.

5. Group V (cinnamic acid -treated group): Eight mice received a suspension of 50 mg/kg cinnamic acid by Oral gavage for 7 days.

The administration of cinnamic acid was done by oral route at the same time during the 7 days (9:00 am). Euthanization was done in the morning on the eighth day by using diethyl ether. Eight mice were sacrificed by cervical dislocation after anesthesia.

Preparation of DSS working solution

The 3% solution of DSS was prepared daily by dissolving 1.68g of DSS in 56 ml distilled water (DW).

Preparation of cinnamic acid working solution

For a dose of 50 mg of cinnamic acid, the stock solution was prepared as suspension with 100mg of poloxamer[12] .The solution was then given to each mouse for seven day. A dose of 50mg/kg (about 0.1 ml of the solution according to animal weight) was given orally by using a 1ml syringe for each mouse for seven consecutive days [13].

For a dose of 25 mg of cinnamic acid, the stock solution was prepared by as suspension with 100mg of poloxamer. The solution was then given to each mouse for seven days. A dose of 25mg/kg (about 0.1 ml of the solution according to animal weight) was given orally by using 1ml syringe for each mouse for seven consecutive days.

Serum collection

Blood was collected by a heparinized microcapillary tube through the retroorbital route. the drained blood (1 – 1.5 ml) was collected in the Eppendorf tube (Figure 2-2) for 20 minutes until blood coagulation occurred. Serum was collected after centrifugation in a cold centrifuge at 3000 rpm for 20 minutes at 4°C and was kept frozen for ELISA kit analysis to detect Tumor necrosis factor α, interleukin 6, nuclear factor-κB(pro-inflammatory cytokines).

Isolation of the colon

After euthanization on the eighth day, the colon was isolated: the distal part was used for histopathological analysis.

Histopathological examination of Colon tissue

The distal colon was isolated and washed with normal saline. Tissue fixation was done by immersing the colon tissue in 10% formalin followed by a water bath. The colon was then dehydrated using consecutive Increasing strengths of ethanol each 1 minute. The colon then was cleaned with Xylene to eliminate alcohol and to provide the colon with some degree of Transparency, and then the tissue was saturated with paraffin wax, heated, and blocked by pouring in embedded models. Blocks were cut by microtone into 5 μm, thick sections, washed in a water bath, Tissue was left in the oven for dewaxing, then stained with hematoxylin and eosin, and then examined using a light microscope by a pathologist[14].

Measurement of various colon injury related parameters

Quantitative analysis of TNF-α

This kit is used to identify the levels of tumor necrosis factor in the colon tissue of mice. The kit was prepared using an enzyme-linked immunosorbent method. The method involves the addition of a mouse's TNF-α antibody to the plate, TNF-α presenting the sample is added and binds to antibodies coated on the wells. The kit then adds a biotin-based antibody to the sample and a Streptavidin-HRP t is added and binds to the Biotinylated TNF-αantibody. Unbound Streptavidin-HRP is then washed away following the incubation step. The resulting color develops due to the concentration of Mouse TNF-α after Substrate solution is added. The reaction is then terminated by the addition of acidic stop solution and absorbance is measured at 450 nm[15].

Quantitative analysis of IL-6

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). It is used to identify the levels of interleukin-6 in the colon tissue of mice. The method involves the addition of a mouse's IL-6 antibody to the plateIL-6 presenting the sample is added and binds to antibodies coated on the wells. The kit then adds a biotin-based antibody to the sample and a Streptavidin-HRP t is added and binds to the Biotinylated IL-6 antibody. Unbound Streptavidin-HRP is then washed away following the incubation step. The resulting color develops due to the concentration of Mouse IL-6 after Substrate solution is added. The reaction is then terminated by the addition of acidic stop solution and absorbance is measured at 450 nm[15].

Quantitative analysis of NF-κB

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). It is used to identify the levels of NF-κB in the colon tissue of mice. The method involves the addition of a mouse's NF-κB antibody to the plate NF-κB presenting the sample is added and binds to antibodies coated on the wells. The kit then adds a biotin-based antibody to the sample and a Streptavidin-HRP t is added and binds to the Biotinylated NF-κB antibody. Unbound Streptavidin-HRP is then washed away following the incubation step. The resulting color develops due to the concentration of Mouse NF-κB after Substrate solution is added. The reaction is then terminated by the addition of acidic stop solution and absorbance is measured at 450 nm[15].

Statistical analysis

The data were analyzed using IBM SPSS statistics v25, means of groups were compared using one-way ANOVA followed by Post hoc (Tukey). All data are expressed as mean ± Standard deviation SD and were considered as significant when the p-value < 0.05.

Result

The effect of Cinnamic acid administration on anti-inflammatory markers in serum for DSS-induced ulcerative colitis in mice is presented in table 1 and figure 1,2 and 3, which showed a significant increase (P<0.05) in TNF-α, IL-6, NF-κB after DSS administration as compared with the distilled water control group. The pretreatment with cinnamic acid at a dose of 50mg/kg produced a significant decrease (P<0.05) in TNF-α, IL-6, NF-κB level as compared with the DSS model group. Furthermore, pretreatment with cinnamic acid 25mg/kg also produce a significant reduction (P<0.05) in TNF-α, NF-κB level but non-significant reduction in IL-6 levels as compared to the DSS model group

Table 1.Effect of cinnamic acid administration on anti-inflammatory markers in colon tissue homogenate for DSS-induced ulcerative colitis in mice.

Group N=8	Level of TNF-α(ng/l) (Mean ± SD)	LEVEL of IL-6 (Pg/ml) (Mean ± SD)	LEVEL of NF-κB (ng/l) (Mean ± SD)
Control group	90.73 ± 10.72	92.87 ± 8.50	1.70 ± 0.14
DSS group	138.15 ± 16.48 *	132.67 ± 6.99 *	2.76 ± 0.26 *
DSS group+ cinnamic acid 50mg/kg	110.78 ± 4.87 #	121.42 ± 8.26	2.07 ± 0.71 #
DSS group +cinnamic acid 25mg/kg	100.25 ± 9.09 #	106.76 ± 23.84 #	1.85 ± 0.35 #
cinnamic acid 50mg/kg	94.16 ± 4.54	103.37 ± 12.84	1.84 ± 0.13

Each value represents mean ± SD
* Is significantly different compared with the control group (P<0.05).
# Is significantly different compared with the DSS group (P<0.05).

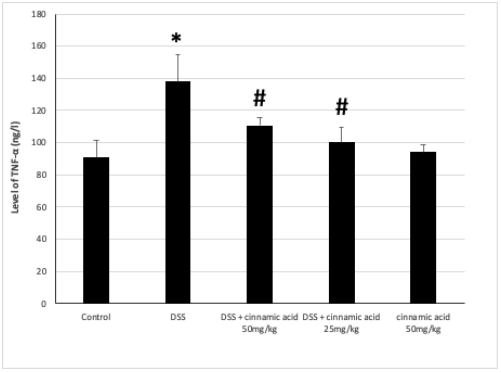


Figure 1. Effect of cinnamic acid administration on TNF-α Levels in serum for DSS-induced ulcerative colitis in Mic

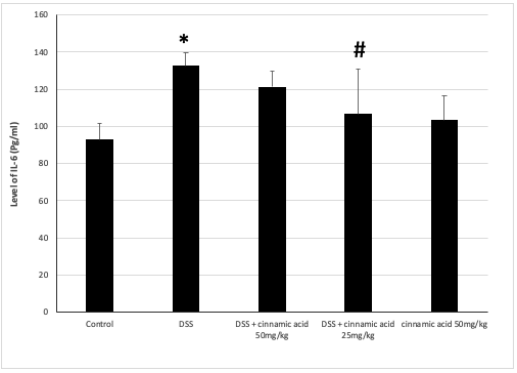


Figure 2. Effect of cinnamic acid on IL-6 Levels in serum for DSS-Induced ulcerative colitis in Mice

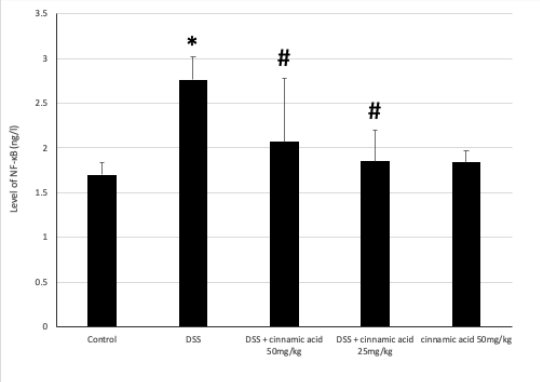
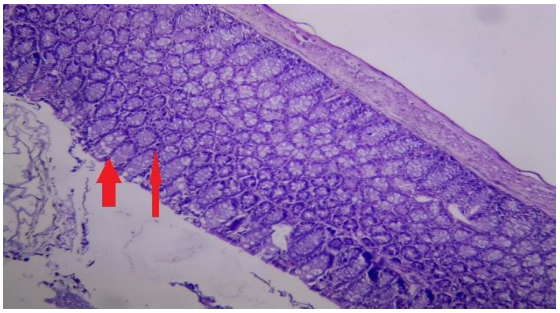


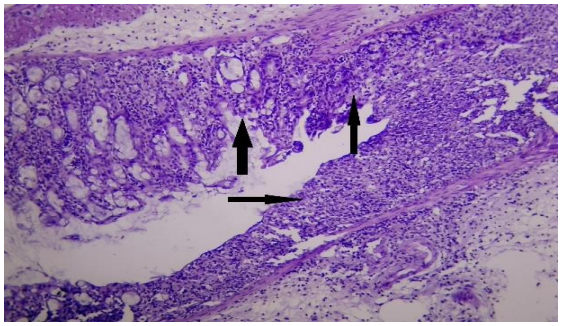
Figure 3. Effect of Cinnamic acid administration on NF-KB Levels in serum for DSS-induced ulcerative colitis in Mice

Histopathology

Histological exanimation with the use of eosin and Hematoxylin was performed blinded. The changes in the samples were examined using an electronic microscope at a magnification of 40 Magnification. Figure 4A illustrates that the control group showed a small amount of inflammatory mast cells. In the model DSS group section (Figure 4B), the presence of inflammatory cells was strongly observed. There were also decreased numbers of goblet cells and colonic crypts. infiltration of inflammatory cells, and extensive submucosal. DSS with cinnamic acid 25mg/kg group (Figure 4C) resulted in the presence of mild inflammatory cells, a slight sloughing of the mucosal epithelium. At (DSS + cinnamic acid 50mg/kg) group (Figure 4D) section look like normal. Cinnamic acid 50mg/kg only treatment section (Figure 4E) showing a slight mild inflammatory cells infiltration.



A



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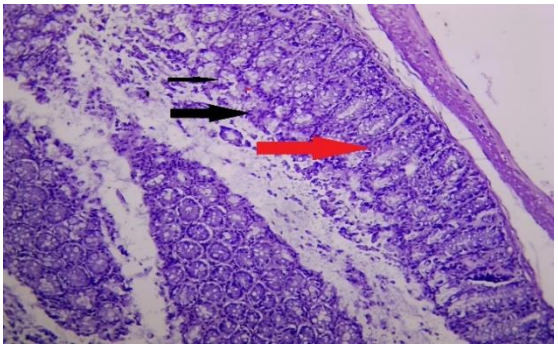

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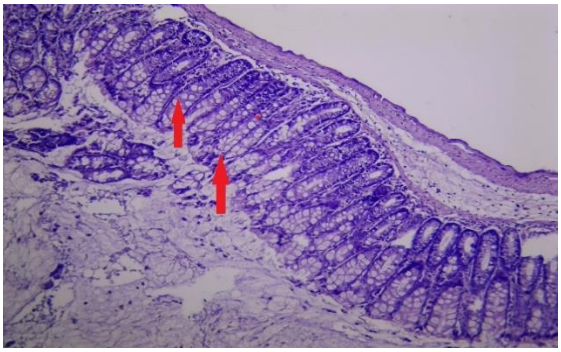

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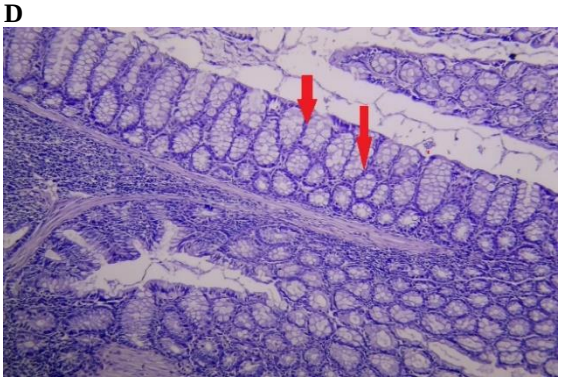

E

Figure 4. Effect of cinnamic acid Histological Assessment of Colon Tissue for DSS-Induced ulcerative colitis in Mice.

Red arrow: mucosa. Black arrow: sloughing of tissue (ulcerative colitis)

Discussion

It has been known that cinnamic acid can potentially benefit the colon by reducing the damage caused by free radicals and inhibiting the oxidation of lipid compounds. Therefore, it can prevent the development of ulcers[16].

The induction of an inflammatory response in the colon has been used as a strategy for the study of the causes of IBD and the development of new drugs. The DSS effect cell barrier by several factors. These include the activation of macrophages, inhibition of epithelial cell overgrowth and the degradation of the DNA replication also, the increase in the release of cytokines and breaking the balance of gut microflora[17]. The effects of the DSS on the colon are usually triggered by the activation of the Th1. However, in chronic and late stages of the inflammation, the Th1 and Th2 activation were

reported. The tissue damage caused by the DSS was mainly caused by the large amount of IL-6 and TNF-α[18].

Currently, there are no studies demonstrating the effects of cinnamic acid on progression of UC. However, its anti-inflammatory effect on different parts of the body were reported by decreasing proinflammatory cytokines. In *Liao et al* (10) study, it was reported that cinnamic acid has significant effect in decreasing both TNF-α and NF-κB levels. Also, in *Fan Song et al* [11] study it was found that cinnamic acid has significant effect in decreasing the level of TNF-α and IL-6. This study is performed to detect cinnamic acid anti-inflammatory effect in UC cases and how it will help in protection or treatment of UC.

In this study it was revealed that the administration of the DSS to the mouse resulted in the development of an inflammatory response by significant increase in serum TNF-α, IL-6 and NF-κB. The presence of certain factors such as the tumor necrosis factor alpha and the interleukin 6 plays a crucial role in the development of inflammatory bowel disease[19]. DSS can alter the function of the cell barrier and the innate immune system. The presence of the TNF- can increase the permeability of the cells and trigger the production of more prostaglandins. In addition, the presence of the IL-6 can stimulate the production of acute-phase proteins and release C3 and C5 as it activates the complement system and neutrophil chemotaxis. These two components are known to trigger the activation of mast cells and release various mediators such as heparin and histamine.as a result , various anti-inflammatory target such as IL-6 and the NF-B have been identified as promising targets for treating IBD[20] .

The presence of the proinflammatory cytokines can trigger the development of apoptosis in UC[21]. Also, the NF-κB can help in the activation of the inflammatory mediators by delivering them to inflammatory mucosae[22]. cyclo-oxygenase-2 (COM2), can stimulate the production of prostaglandins involved in the metabolic pathway of IBD[23]. the production of leukotrienes increases inflammatory processes, the LTB4 can also trigger the activation of the neutrophil-dependent hyperalgesia and the transcription of various cytokines. In addition, inflammatory bowel disease is a complex disorder that is caused by a deregulated immune response that leads to the overproduction of nitric oxide lead to high levels of nitric oxide generation[24] .

The tumor necrosis factor is produced by various cells, such as macrophages, monocytes, and natural killer cells[25,26]. It is known to play a role in the inflammatory response and the development of various physiological and pathological conditions[27].It is also known that the presence of the tumor necrosis factor can promote the development of pro-apoptotic signaling by binding to the type I TNF receptor[28].

In a study, the levels of the tumor necrosis factor were significantly elevated in the DSS group compared to the control group. It was shown that the presence of cinnamic acid can ameliorate the effects of DSS on the development of an inflammatory response in the mouse. The decrease in the serum levels of various pro-inflammatory cytokines was also observed.

It is also known that the presence of the tumor necrosis factor can promote the development of pro-apoptotic signaling by binding to the type I TNF receptor. In a study, the levels of the tumor necrosis factor were significantly elevated in the DSS group compared to the control group. It was shown that the presence of cinnamic acid can ameliorate the effects of DSS on the development of an inflammatory response in the mouse. The decrease in the serum levels of various pro-inflammatory cytokines was also observed, at least partly due to its inhibition on the inflammatory factors

It's very important that drugs that block the effects of certain metabolites in various diseases such as asthma, psoriasis, ulcerative colitis and arthritis are developed for the treatment of IBD and other conditions[29,30].

The property of cinnamic acid as anti-inflammatory influenced to a great extent by the substituents on the aryl ring and the double bond [29],also have been related to the inductive effects of α, β-unsaturated carbonyl compounds and . In most tissues and cells, the overexpression of nitric oxide two leads to the production of nitric oxide, which then increases the activity of the cyclooxygenase two. This process also helps in the formation of a peroxinitrite anion[31]. The downstream target of the NOS2 is the heme oxygenase-1 (HO1). This enzyme can convert heme into various compounds, such as carbon monoxide, biliverdin, and iron. It also inhibits the development of inflammation and immune responses[31].

Inhibition of the activation of the Src/spleen-tyrosine kinase by C. ramulus compounds can reduce the expression of NF-κB[32]. The compounds in the C. ramulus family exhibited anti-inflammatory effects when they were able to suppress the expression of nitric oxide. The compounds exhibited anti-inflammatory properties by preventing the production of certain chemicals, such as nitric oxide and cyclooxygenase two. These substances could be useful in the treatment of inflammatory diseases by blocking the production of these chemicals. The extract of cinnamon can also decrease the levels of a certain type of tumor necrosis factor in the serum [33] .

Conclusion

The treatment with cinnamic acid significantly decreased the levels of DSS induce pro-inflammatory cytokines. This finding supports the idea that the use of this substance could be used as a potential therapy for patients with ulcerative colitis.

Funding

We hereby confirm that no external funding or financial contributions were received or utilized in the preparation, research, writing, or publication of this article.

Conflicts of Interest

The authors declare that there is no conflict of interest

Ethics Statements

This article was approved by the ethical committee of the College of Pharmacy/University of Baghdad

Author Contribution

Author roles: Maysam Ameer Hussein Data curation, Formal analysis, acquisition, Investigation, Project administration, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. Munaf H. Zalzala Conceptualization, Methodology, Project administration, Validation, Writing – review & editing.

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