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# Colon COX-2, IL-6, TNF-α, oxidant and antioxidant defense system as a potential target in chemoprevention of dextran sulfate sodium salt-induced ulcerative colitis in rats: Role of aloin

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## ABSTRACT

*This study designed to investigate the anti–inflammatory effect of aloe component (aloin) on inflammatory mediators and oxidative stress in dextran sulfate sodium salt induced ulcerative colitis (UC) in rats. Twenty-four albino rats divided into four equal groups of eight rats each. Group 1: (normal control) received no drugs , group 2: (ulcerative colitis) rats received dextran sulfate sodium salt 3% in drinking water, group 3: (aloin) rats administered orally with 1ml of a loin 0.1% daily for 3weeks, group 4: (aloin +UC) rats with ulcerative colitis orally treated with (aloin 0.1%), daily for 3weeks . The obtained results revealed that, administration of aloin to rats with ulcerative colitis showed non-significant change on serum total cholesterol and TG concentrations, and markedly increased the reduced HDL-C level. On the other hand, elevated level of COX-2, IL-6, MDA and TNF-α in UC rats were significantly reduced, with significant increase of the reduced level of GSH. Results revealed that aloin showed high efficacy to normalize UC tissues and may considered as potential treatment for UC and other inflammatory bowel disease.*

**Keywords:** aloin, ulcerative colitis, inflammatory mediators, histopathology.

## 1. INTRODUCTION

Ulcerative colitis is an inflammatory bowel disease (IBD) that causes long lasting inflammation and ulcers (sores) in the digestive tract. Ulcerative colitis affects the most inner lining of large intestine (colon) and rectum. In fact, the exact cause of ulcerative colitis is unknown. Researchers believe that factors such as over active intestinal immune system, genes, and environment may play role in causing ulcerative colitis [1].

Induced intestinal inflammation are one of the most commonly used models because they are simple to induce, the onset, duration, and severity of inflammation are immediate and controllable. Dextran sulfate sodium salt (DSS) induced colitis are well-established animal models of mucosal inflammation that have been used for over 2 decades in the study of IBD pathogenesis and preclinical studies. The DSS-induced colitis model has some advantages when compared to other animal models of colitis. For example, an acute, chronic, or relapsing model can be produced easily by changing the concentration of administration of DSS. Moreover, dysplasia that resembles the clinical course of human UC occurs frequently in the chronic phase of DSS-induced colitis [1 and 2].

*Aloe vera* is known for their nutraceutical and cosmeceutical properties including anti-viral, anti-bacterial, laxative, antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-allergic, immuno-stimulant, UV protecting activity and so on [4 - 6]. The

triterpenoidlupeol and the steroids cholesterol, campestrol and b-sitosterol were all found in whole leaf extracts of aloevera [7 and 8] Lupeol, campestrol and b-sitosterol were found to be significantly anti-inflammatory in wounded mice [9].

Aloin is known to be hydrolyzed by the esterases secreted by intestinal microflora [10].Once the c-glycosides has been hydrolyzed, it forms the aloe-emodin, anthrone which is further auto-oxidized to the quinine, aloe-emodin. Since aloin contain a polyphenolic structure, these compounds may also responsible for the reported anti-inflammatory effect of aloe [11 and 12]. Accordingly, this study was performed to investigate the therapeutic effect of aloin on DSS-induced ulcerative colitis in rats.

## 2. MATERIALS AND METHODS

### 2.1-Chemicals:

Dextran sulfate sodium salt: (DSS) extracted from *Leuconostoc spp*. with average molecular weight of 500,000 ,and Aloin: from curacao aloe, molecular weight 418.29, have been obtained from (sigma-Aldrich comp. for trading chemicals).

### 2.2-Experimental design:

Twenty-four white albino rats of 5-7 weeks old and weighting 100-120gm were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and fresh, clean drinking water was supplied ad-libitum.

Induction of ulcerative colitis: ulcerative colitis has been induced in rats with dextran sulfate3% orally administered in drinking water for 7 days [13] . Dosage of aloinorally administered at dose 1ml of aloin 0.1%, daily for 3weeks [14].

### 2.3-Animal groups:

Rats were randomly divided into four equal groups , 8 animals each , placed in individual cages and classified as following: group1(control group), feed standard pellet diet and clean drinking tab water. Group2 (UC induced group): Rats were orally received dextran sulfate3% in drinking water . Group3 (aloin administered group): rats received 1ml a loin 0.1% orally administered by gavage for 3weeks. Group 4:(aloin + ulcerative colitis group), rats with ulcerative colitis orally and daily administered with 1ml of a loin 0.1% for 3weeks.

### 2.4-Sampling:

Blood samples and colon tissues were collected from all animal groups at the end of the experiment.

*2.4.1- Blood samples:*

Blood samples were collected after overnight fasting in dry, clean and screw-capped tubes. Serum was separated by centrifugation at 4000 r.p.m for 15 min. the clear serum was received in dry, sterile sample tubes and kept in a deep freeze at -20° C until used for subsequent biochemical analysis. All sera analyzed for the following parameters: total cholesterol, HDL-C , TG , TNF-α and IL-6 .

*2.4.2-Tissue samples (colon tissue):*

At the end of the experiment, rats of each group were sacrificed by cervical decapitation. The abdomen was opened and the colon specimen was quickly removed and opened gently using a scrapper, cleaned by rinsing with ice-cold isotonic saline to remove any blood cells, clots and scraps of food, then blotted between 2 filter papers and quickly stored in a deep freezer at (-20 °C) for subsequent biochemical analysis. Briefly, colon tissues were divided into appropriate portions, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant were used for the determination of the following parameters: GSH , COX-2 ,MDA.

### 2.5-Histopathological examination:

Washing colon tissues was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidgemicrotome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain for examination through the light electric microscope (15).

### 2.6-Biochemical Analysis:

Serum total cholesterol l, HDL-C, TG were determined according to the methods described by [16 - 18] and TNF-α and IL-6 estimated by ELISA kit supplied by R &D system Quantitative, USA.also, colon tissue Malondialdehyde (MDA) ,COX-2 and reduced glutation (GSH) were determined according to the methods described by [19].

### 2.7-Statistical analysis:

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA). All analysis performed using the statistical package for social science (SPSS, 2009). The Values were considered statistically significant when $p \leq 0.5$

**Table 1:** Effect of aloin treatment on serum total cholesterol, Triglyceride ,and HDL-C levels in DSS induced ulcerative colitis in rats and their control.

| Animal groups | Cholesterol(mmol/dl) | TG (mgl/dl ) | HDL (mg/dl ) |
|---|---|---|---|
| Control | 138.4 ± 12.3 | 108.9±6.6 | 57.1±4.0$^{b}$ |
| Ulcerative colitis(uc) | 142.7 ± 11.5 | 109.8±13.2 | 21.9±2.5$^{a}$ |
| Aloin | 139.4± 9.0 | 111.1±9.5 | 56.4±3.3$^{b}$ |
| UC+Aloin | 131.0 ± 11.8 | 98.9 ± 4.1 | 59.8 ± 6.4$^{b}$ |

Data are presented as (mean±SD). SD: standard deviation mean values with different superscript letters in the same column are significantly different at (*p≤0.5*).

**Table 2:** Effect of aloin treatment on serum COX-2 activity ,IL6 ,and TNF-α levels in DSS induced ulcerative colitis in rats and their control.

| Animal groups | COX2 (u/g ) | IL6 (pg/mL ) | TNFa (pg/ml ) |
|---|---|---|---|
| Control | 1.1±0.1$^{b}$ | 34.7±2.4$^{b}$ | 33.2±4.7$^{b}$ |
| Ulcerative colitis (uc) | 13.1±1.7$^{a}$ | 149.0±12.1$^{a}$ | 122.9±3.9$^{a}$ |
| Aloin | 1.2±0.08$^{b}$ | 30.3±5.3$^{ab}$ | 34.5±8.3$^{ab}$ |
| UC+Aloin | 1.0 ± 0.02$^{b}$ | 31.0 ± 3.3$^{b}$ | 37.3 ± 4.4$^{b}$ |

Data are presented as (mean±SD). SD: standard deviation mean values with different superscript letters in the same column are significantly different at (*p≤0.5*).

**Table 3:** Effect of aloin treatment on MDA level and GSH level in DSS induced ulcerative colitis in rats and their control.

| Animal groups | MDA (mmol/mg ) | GSH (mmol/mg ) |
|---|---|---|
| Control | 1.1±0.1$^{b}$ | 56.4±2.5$^{b}$ |
| Ulcerative colitis (uc) | 21.1±8.1$^{a}$ | 17.8±2.2$^{a}$ |
| Aloin | 1.2±0.05$^{b}$ | 59.5±3.1$^{b}$ |
| UC+Aloin | 0.9 ± 0.06$^{b}$ | 57.1 ± 4.3$^{b}$ |

Data are presented as (mean±SD). SD: standard deviation mean values with different superscript letters in the same column are significantly different at (*p≤0.5*).

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of aloin treatment on some serum and colon tissue parameters of DSS-induced ulcerative colitis in rats.

The obtained results in table (1) revealed that ,administration of DSS induced UC in rats exhibited non-significant change in serum total cholesterol and TG concentrations and significantly decreased HDL-C level when compared with normal control groups. Treatment with aloin to DSS induced ulcerative colitis in rats showed non-significant change in serum total cholesterol and TG concentrations, and increase markedly HDL-C level when compared with ulcerative colitis non-treated group.

The results presented in table (2) showed that administration of DSS induced in rats exhibited a significant increase in serum level of COX-2, IL-6, and TNF-α when compared with normal groups. Treatment with aloin to DSS induced ulcerative colitis in rats significantly reduced elevated level of TNF-α, IL6, and COX-2 gene expression.

The obtained results in table (3) revealed that administration of DSS induced UC in rats exhibited a significant increase in colon tissue MDA, and significantly decreased GSH concentration when compared with normal group. Treatment with aloin to DSS induced ulcerative colitis in rats significantly reduced the elevated level of MDA , and markedly increase the reduced GSH level in colon tissue.

*3.2-Histopathological findings*:

Histopathological studies on colon tissue sections of control group showed no histological alteration were observed, while rats group which treated with DSS showed massive numbers of inflammatory cells infiltration in the colon mucosal and sub mucosal layers. Treatment of DSS-induced UC rats byaloin show focal inflammatory cells in the base of the mucosa.

Inflammatory bowel disease (IBD) is a complex multifactorial disease [20]. It commonly refers to ulcerative colitis (UC) and Crohn's disease (CD), the two chronic conditions that involve inflammation of the intestine. Despite recent advances in treatment, there remains a need for a safe, well-tolerated therapy with a rapid onset, and increased capacity for maintaining long-term remission [21].

Aloe is widely used in the food product and pharmaceutical industries due to its biological functions of anti-inflammatory activity [22], acceleration of wound healing [23], and protective effect against liver injury [24], although it is not well

understood which activity is related to which component. Inflammatory bowel disease (IBD),Crohn's disease and ulcerative colitis (UC) are frequent illnesses in many parts of the world, especially in industrialized countries [25].

Treatment with aloin to DSS induced ulcerative colitis in rats significantly reduced elevated serum total cholesterol and TG concentrations, with increase the HDL-C. These results are nearly similar to those reported by [26], which said that the treatment effectively decreased elevations in serum levels of total cholesterol and TG, and increased HDL-C level caused by DSS.

DSS as a model for studying colitis-associated carcinogenesis [7] .Kanneganti, et al. who investigate the validated DSS model by using different therapeutic agents for human IBD and showed that DSS-induced colitis can be used as a relevant model for the translation of mice data to human disease [28 and 29]. Intestinal microflora and their products have been implicated in the pathogenesis of human IBD [20,30 and 31 ] and in several animal models [32]. The importance of the intestinal flora is directly supported by studies of somewhere colitis is not observed when they are reconstituted with bacteria that are considered normal constituents of luminal flora It has been demonstrated that intestinal flora is implicated in the pathogenesis of DSS colitis in mice. First who suggested contribution of colonic bacteria or their products in the development of colitis in this model were Okayasu et al. They observed increased numbers of Enterobacteriaceae, Bacteroidaceae, and Clostridium spp. in the colons of mice affected by DSS colitis [33].

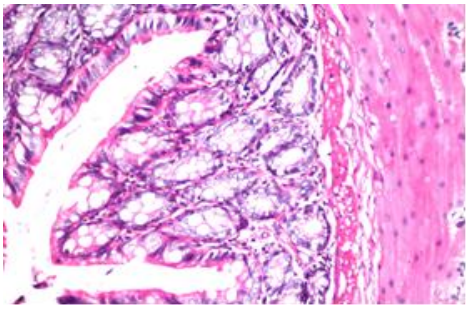

**Figure 1:** Control group: There was no histopathological alteration in the colon.

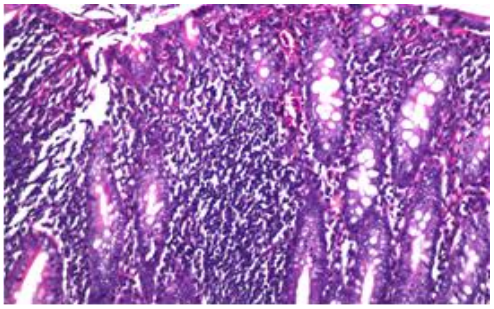

**Figure 2:** UC group: The mucosal and submucosal layers showed massive numbers of inflammatory cells infiltration in the colon.

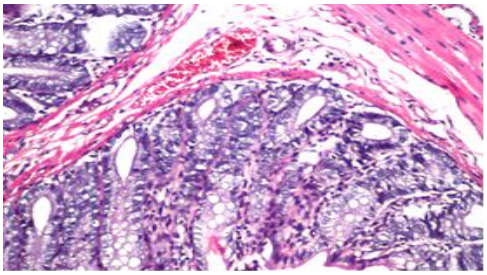

**Figure 3:** Aloin group: There was no histopathological alteration**.**

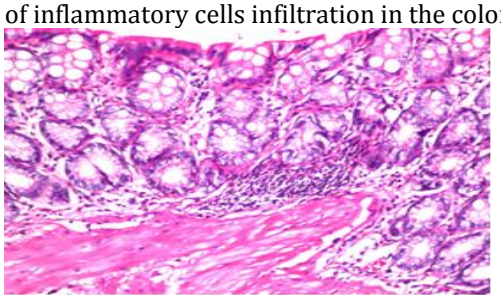

**Figure 4:** UC treated with aloin: Focal inflammatory cells infiltration was noticed in the base of the mucosa.

DSS-induced breakdown of mucosal epithelial barrier function allows the entry of luminal antigens and microorganisms into the mucosa resulting in overwhelming inflammatory response Numerous inflammatory mediators have been implicated in the pathogenesis of human IBD .Changes in production of inflammatory mediators in DSS-treated mice were investigated during different phases of colitis, in the serum and or colon and by different methods. Increased expression of different inflammatory mediators (TNF-$\alpha$) was observed as early as the first days of DSS treatment [34] .The production of these inflammatory mediators increased progressively during DSS treatment. Different profile of inflammatory mediators in acute and chronic phase of DSS colitis was demonstrated as recorded by elevated levels of IL-6 [35]. Progressive up regulation was observed with increasing dosage of DSS[36].These inflammatory mediators not only play a role in the pathogenesis of DSS-induced colitis but are important as intervention targets against colitis as excellently described by [37], Cytokine profile in DSS colitis correlates with clinical and histological parameters as well as barrier properties. Human and animal studies support the idea that TNF-α and interleukins are important pathological mediators of IBD [38 and 39]. In humans with IBD, approximately two-thirds of patients respond to anti-TNF-α treatments [40], and intestinal inflammation is attenuated significantly by anti-interleukins and or anti-TNF-α monoclonal antibodies in mice [41,42].

TNF-α and interleukins as IL-6 mRNA expressions in the colon of DSS-exposed rats are dramatically increased compared to non-colitic rats, suggesting that immune cells are attracted to the site of inflammation. Dietary aloin, supplementation significantly decreased these inflammatory cytokine expressions in a dose-dependent manner. In this study, aloe components clearly suppressed the expression of TNF-α and IL-6 in the colon [43]. This results are similar also to those which reported by [26].

COX-2 can be activated to produce excessive PGE2, an important inflammatory mediator in IBD [44]. COX-2 is pro-inflammatory protein that plays pivotal role in mediating inflammation and contribute to chemical-induced inflammation in mice [45]. In the present study aloin was found to be significantly down regulating COX-2 expression in colon.

COX-2 enzymes , which catalyze prostaglandin biosynthesis , has become an important target for the discovery and development of new anti-inflammatory agents [43]. Aloin structure-activity study has indicated that more than 2 hydroxyl groups on the B ring were important for suppression of COX-2 transcription activity [46]. The current study showed that, the value of MDA level, a marker of oxidative stress, was significantly higher in the DSS group. Meanwhile in treatment groups the MDA levels in the colonic tissue markedly decreased compared with the DSS groups . GSH plays a common role in cellular resistance to oxidative damage as a free radical scavenger as protein-bound GSH and by generation of ascorbate and/or tocopherol in liver [47]. Treatment cause a significant increase in GSH level .This results were nearly similar to those reported by Manikandan et al. [48] who showed that, curcumin reversed the effect of gentamycin, by significantly increasing GSH activity in the kidney tissue.

## 4. CONCLUSION

Administration of aloin to rats with ulcerative colitis markedly increased the reduced HDL-C level. On the other hand, elevated level of COX-2, IL-6, MDA and TNF-α in UC rats were significantly reduced, with significant increase of the reduced level of GSH. Results suggest that aloin showed high efficacy to normalize UC tissues and may considered as potential treatment for UC and other inflammatory bowel disease.

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