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SHORT COMMUNICATION

Efficacy of Lactoferrin with Dexamethasone Sodium on Hematological Profile Against Acetic Acid Induced Colitis in Rats

Lavudya Naveen[1*], Ashok Kumar Devarasetti[2], Akkaladevi Jayasri[1], Alla Gopala Reddy[3] and Mohammed Shaz Murtuza[1]

[1]Department of Veterinary Biochemistry, College of Veterinary Science, Rajendranagar,PV Narasimha Rao Telangana Veterinary University, Hyderabad, INDIA

[2]Department of Veterinary Biochemistry, College of Veterinary Science, Mamnoor, PV Narasimha Rao Telangana Veterinary University, Hyderabad, INDIA

[3]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, PV Narasimha Rao Telangana Veterinary University, Hyderabad, INDIA

[*]Corresponding author: L Naveen; E-mail: lavdyanaveennaik@gmail.com

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ABSTRACT

Lactoferrin (Lf) is a member of transferrin family. It is a glycoprotein that binds mainly to iron. Lf is present in exocrine secretions and it has been reported to have various defensive function. The present study was aimed to evaluate the hematological parameters when lactoferrin (@ 200 mg/kg b.wt.) was administered orally in acetic acid-induced colitis (4% @ 2 ml single dose intra-colonic) in rats. Hematological parameters revealed that, the means of Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC) were improved significantly ($P < 0.05$) in Lf treated group. The results were suggested that, Lf has positive effect on hematological profile on acetic acid - induced colitis in rats. Therefore, Lf is a promising potential therapeutic agent for the treatment of colitis.

HIGHLIGHTS

- Total leucocyte count, neutrophil and eosinophil count increased significantly in rats treated with 4% acetic acid.
- Lactoferrin-treated groups had a faster return to near-normal haematological parameters on the 14th day.

Keywords: Lactoferrin, Colitis, Dexamethasone sodium and Hematological profile

Lactoferrin (Lf), an iron-binding glycoprotein found in milk, exocrine secretion and serum etc., belongs to the transferrin family (Farid *et al*., 2019). Human and bovine milk are the most common sources of LF. The amount of LF in milk varies greatly depending on lactation stage and species (Wang *et al*., 2019). The milk samples of Indigenous cow breeds are rich in Lf compared to crossbred cows (Ashok Kumar *et al*., 2018). Lf was also isolated from indigenous breeds of cattle (Shaz Murtuza *et al*., 2021). Lactoferrin shows to have anti-inflammatory, anti-cancer, antibacterial, antioxidant, and immunomodulatory properties (Superti *et al.,* 2020). LF is a vital nutrient for maintaining host immunity, as well as functioning as an antibacterial and antiviral agent (Kell *et al*., 2020). Various studies have shown that Lf exibits anti-inflammatory property by increasing expression of interleukin-11 (Kuhara *et al*. 2014). Intestinal inflammation was dramatically reduced in pigs who were fed Lf (Cooper *et al*., 2014).

Ulcerative Colitis (UC) is a recurrent inflammatory bowel disease (IBD) characterised by long-term inflammation of

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the colon and rectum linings (Owusu *et al*., 2020). UC is an idiopathic inflammatory bowel disease that damages the colonic mucosa and is characterised by diarrhoea, stomach pain, and weight loss (Osafo *et al*., 2018). Although the cause of inflammatory bowel disease (IBD) is unknown, it appears that an abnormal response of the mucosal innate immune system to luminal bacteria may trigger inflammation, which is perpetuated by cellular immunity dysregulation and imbalances between proinflammatory cytokines such as TNF-α, IFN-γ, IL-1β, IL-6, and IL-12 and anti-inflammatory cytokines such as IL-4, IL-10, and IL-11 (Kumar *et al*., 2018).

In ulcerative colitis, exogenous glucocorticoids are commonly utilised as anti-inflammatory drugs and are the first line of treatment (Dubois-Camacho *et al.,* 2017). The present work was done to study the blood cell profile on oral feeding of lactoferrin alone, as well as combination of dexamethasone sodium with lactoferrin on acetic acid induced colitis in rats by analysing and comparing various hematological parameters at different time points.

The current investigation was carried out on healthy adult female Wistar rats weighing 200-250 gm and of uniform age group (3 months). The rats were cared for according to CPCSEA guidelines.

The protocol used in this investigation was approved by the Institutional Animal Ethics Committee (IAEC) and follow the rules for the care and use of laboratory animals, with IAEC permission number 22/24/CVSc/Hyd/IAEC - Wistar Rats/12.06.2021. Colitis was induced in rats using a single dosage of 2 mL of acetic acid (@ 4 percent v/v) administered intra-colonically. Rats were given full access to water after being deprived for 24 hours. A plastic paediatric catheter with an outside diameter of 2 mm was inserted 8 cm deep into the colon through anal route, under light anaesthesia condition. All the rats under this study were infused 2 mL of 4 percent diluted acetic acid and held head-down position for 30 seconds before the anal discharge fluid was collected (Owusu *et al*., 2020).

Group 1: Control; Group 2: Acetic acid induced colitis; Group 3: Acetic acid induced colitis + Intraperitoneal injection of dexamethasone sodium (2 mg/kg body weight for 14 days); Group 4: Acetic acid induced colitis + Oral feeding of lactoferrin (200 mg/kg body weight for 14 days); Group 5: Acetic acid induced colitis + Intraperitoneal injection of dexamethasone sodium (1 mg/kg body weight for 14 days) + Oral feeding of lactoferrin (100 mg/kg body weight for 14 days). Blood was collected from retro-orbital plexus into EDTA coated vacutainers for blood cell profile. Fully automated hematology analyzer (Medsource ozone Bio chemicals Pvt. Ltd.) was used for hematological parameters.

The data were statistically analysed using the statistical programme for social sciences (SPSS) version 25.0 and one-way ANOVA. Duncan's multiple comparison test was used to assess for differences between means, with a significance level of $P<0.05$.

The mean total leucocyte count ($x10^3/\mu l$) (16.11 ± 0.92 and 16.18 ± 0.93, respectively), mean neutrophil count (%) (9.33±0.16 and 9.43±0.16, respectively) and mean eosinophil count (%) (7.60 ± 0.11 and 7.7 ± 0.11, respectively) in group 2 were significantly ($P < 0.05$) increased as compared to group 1 and treatment groups on 7th and 14th day. The mean lymphocyte count (%) (52.80±1.05 and 52.75±1.04, respectively) and mean monocyte count (%) (2.25±0.12 and 2.16±0.11, respectively) in group 2 was significantly ($P < 0.05$) decreased as compared to group 1 and treatment groups on 7th and 14th day. In the treatment groups 3, 4 and 5, there was a significant ($P<0.05$) improvement in all the hematological parameters when compared to group 2 on 7th and 14th day (Table 1-5).

Table 1: Mean values of TLC parameters in experimental groups of rats.

Group	TLC ($x10^3/\mu l$)		
	0th day	**7th day**	**14th day**
1. Control	11.19± 0.45^a	11.19± 0.54^c	11.27± 0.55^c
2. AA (colitis group)	11.17± 0.26^a	16.11±0.92^a	16.18± 0.93^a
3. AA+Dex (2 mg/kg, I/P)	11.34± 0.40^a	14.17± 0.71^b	14.07± 0.71^b
4. AA+Lf (200 mg/kg)	11.18± 0.02^a	13.41± 0.28^b	13.32± 0.27^b
5. AA+Dex (1 mg/kg, I/P)+Lf (100 mg/kg)	11.17± 0.01^a	11.35± 0.47^c	11.25± 0.47^c

Values are Mean ± SE (n=6); One way ANOVA (SPSS);

Means with different alphabets as superscripts differ significantly ($p<0.05$).

Table 2: Mean values of lymphocyte parameters in experimental groups of rats

Group	Lymphocyte (%)		
	0th day	**7th day**	**14th day**
1. Control	72.87± 0.31[a]	72.88± 1.98[a]	73.28 ± 1.96[a]
2. AA (colitis group)	72.78± 0.26[a]	52.80 ± 1.05[d]	52.75 ± 1.04[d]
3. AA + Dex (2 mg/ kg, I/P)	72.85± 0.14[a]	60.00± 2.03[c]	60.27 ± 2.02[c]
4. AA+Lf (200 mg/kg)	72.86± 0.28[a]	65.20± 1.68[b]	65.33 ± 1.68[b]
5. AA+Dex (1 mg/kg, I/P) + Lf (100 mg/kg)	72.86± 0.26[a]	71.93± 0.70[a]	72.03 ± 0.70[a]

Values are Mean ± SE (n=6); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly (p<0.05).

Table 3: Mean values of Neutrophils (%) in experimental groups of rats

Group	Neutrophils (%)		
	0th day	**7th day**	14th day
1. Control	7.73± 0.31[a]	7.74± 0.10[d]	7.84 ± 0.10[d]
2. AA (colitis group)	7.69± 0.91[a]	9.33±0.16[a]	9.43 ± 0.16[a]
3. AA + Dex (2 mg/ kg, I/P)	7.74± 0.25[a]	8.91±0.16[b]	8.83 ± 0.15[b]
4. AA + Lf (200 mg/ kg)	7.75± 0.01[a]	8.36± 0.08[c]	8.26 ± 0.08[c]
5. AA + Dex (1 mg/kg, I/P) + Lf (100 mg/kg)	7.73± 0.26[a]	7.95± 0.11[d]	7.85 ± 0.11[d]

Values are Mean ± SE (n=6); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly (p<0.05).

Table 4: Mean values of Monocytes (%) in experimental groups of rats

Group	Monocytes (%)		
	0th day	**7th day**	**14th day**
1. Control	5.23± 0.31[a]	5.26 ± 0.14[a]	5.36 ± 0.14[a]
2. AA (colitis group)	5.23± 0.17[a]	2.25 ± 0.12[d]	2.16 ± 0.11[d]
3. AA + Dex (2 mg/ kg, I/P)	5.22± 0.25[a]	3.26 ±0.10[c]	3.36 ± 0.10[c]
4. AA+Lf (200 mg/kg)	5.25± 0.12[a]	3.91 ±0.18[b]	4.01 ± 0.18[b]
5. AA+Dex (1 mg/kg, I/P) + Lf (100 mg/kg)	5.23± 0.23[a]	5.04 ± 0.22[a]	5.14 ± 0.22[a]

Values are Mean ± SE (n=6); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly (p<0.05).

Table 5: Mean values of Eosinophils (%) in experimental groups of rats

Group	Eosinophils (%)		
	0th day	**7th day**	**14th day**
1. Control	2.51± 0.31[a]	2.54 ± 0.10[a]	2.64 ± 0.10[a]
2. AA (colitis group)	2.50± 0.01[a]	7.60 ± 0.11[e]	7.7 ± 0.11[e]
3. AA + Dex (2 mg/ kg, I/P)	2.52± 0.10[a]	4.56 ± 0.11[b]	4.46 ± 0.11[b]
4. AA +Lf (200 mg/kg)	2.50± 0.26[a]	4.10 ± 0.10[c]	4.03 ± 0.08[c]
5. AA+Dex (1 mg/kg, I/P) + Lf (100 mg/kg)	2.49± 0.26[a]	3.16 ± 0.09[d]	3.06 ± 0.09[d]

Values are Mean ± SE (n=6); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly (p<0.05).

The effect of acetic acid induced colitis in rats on hematological parameters in this study revealed a significant ($P < 0.05$) higher values of TLC, neutrophil count and eosinophil count in group 2 compared to group 1 and other treatment groups (3, 4 and 5). Tissue injury may cause an imbalance in haematological parameters such as total leukocyte count and differential leukocyte count. These results were correlated with findings of Osafo *et al*. (2019) and Owusu *et al*. (2020). The lower lymphocyte count in group 2 could be due to the immunosuppressive effects of colitis on the bone marrow, which restrict T cell function. (Olamilosoye *et al*., 2018). A significantly ($P < 0.05$) lower values of TLC, neutrophils and eosinophils were observed in the group treated with dexamethasone sodium when compared to colitis control group, which could be due to immunosuppressive action of dexamethasone sodium that acts by decreasing the neutrophil margination pool in the blood vessels (Roth *et al*., 1982 and Sale compos *et al*., 2017). The treatment group 4 revealed a significant ($P < 0.05$) restoration in the hematological parameters by lactoferrin. Lactoferrin's mechanism comprises a number of components that influence cellular immune responses in *in-vivo* inflammatory models. These results are closely related to the findings of Togawa *et al*. (2002) and Farid *et al*. (2019). Greater restoration of hematological parameters to near normally on 7th and 14th day was observed in group 5, which might be due to additive effect of dexamethasone sodium and lactoferrin.

CONCLUSION

Finally, the study discovered that acetic acid-induced colonic injury results in the production of inflammatory

mediators and the synthesis of free radicals, which leads to the breakdown of the intestinal mucosal barrier. According to the results of the present study revealed that, treatment with lactoferrin showed better amelioration compared to dexamethasone sodium alone. But, the combined beneficial effects of lactoferrin and dexamethasone sodium was found to possess protective action against acetic acid induced colitis in rats. Hence, the combined protective action of lactoferrin and dexamethasone sodium against acetic acid induced colitis in rats could be the best promising therapeutic nutraceutical compound to improve hematological profile in this study.

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