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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF MESALAMINE BY GRBC STABILISATION ASSAY METHOD

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ABSTRACT

The objective of the study is to evaluate the anti-inflammatory potential of Mesalamine using the Goat Red Blood Cell membrane stabilization assay. Inflammation is a complex biological response to harmful stimuli and NSAIDs play a crucial role in managing inflammatory conditions. In this study, the GRBC membrane stabilization assay was employed to assess the protective effect of Mesalamine against hypotonic stress-induced hemolysis. The percentage membrane protection and percentage hemolysis were calculated to determine its stabilizing effect. The results demonstrated significant membrane stabilization activity, indicating that Mesalamine effectively prevents RBC lysis and helps maintain membrane integrity under inflammatory conditions. This study provides valuable insights into the anti-inflammatory mechanism of Mesalamine and supports its therapeutic role in inflammation management.

KEYWORDS: Mesalamine, Anti-inflammatory activity, GRBC membrane stabilization, Hemolysis, NSAIDs.

INTRODUCTION

Mesalamine (5-aminosalicylic acid, 5-ASA) is an anti-inflammatory drug primarily used to treat inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease. It works by inhibiting pro-inflammatory mediators, such as cytokines and leukotrienes, thereby reducing inflammation in the gastrointestinal (GI) tract.[1]

The drug exerts a local effect on the colonic mucosa, with minimal systemic absorption, making it a preferred treatment for mild-to-moderate cases of ulcerative colitis. Various formulations, including oral, rectal suppositories, enemas, and controlled-release tablets, have been developed to improve drug delivery and adherence.[2]

GRBC Membrane Stabilization Activity refers to a laboratory assay used to evaluate the anti-inflammatory properties of substances. The method relies on the principle that many inflammatory processes are associated with the destabilization or lysis of cell membranes, and compounds with anti-inflammatory properties can help stabilize these membranes, preventing damage.

PRINCIPLE OF GRBC

The GRBC membrane is similar to the lysosomal membrane in structure. During inflammation, lysosomal enzymes are released, causing tissue damage. If a compound can stabilize the GRBC membrane, it may similarly stabilize lysosomal membranes, indicating potential anti-inflammatory activity.

The GRBC membrane stabilization assay is primarily used in pharmacological research to assess the potential anti-inflammatory properties of drugs, natural compounds, or plant extracts.

EXPERIMENTAL APPARATUS

1. Electronic Balance Samson
2. Centrifuge Alco India
3. Incubator Rotek

REAGENTS AND MATERIALS

1. 10 % v/v GRBC suspension
2. Alsever's solution
3. Phosphate buffer solution (pH 7.4)
4. Hyposaline solution (Prepared by dissolving 0.25 g NaCl in phosphate buffer solution and make up to 20 ml.)
5. Isosaline solution (prepared by dissolving 0.85 g NaCl in phosphate buffer and make up to 20 ml.)

EXPERIMENTAL PROCEDURE

Preparation of GRBC suspension

Mix fresh goat blood with alsever's solution

Centrifuge at 3000 rpm for 10 minutes

Wash packed cell with isosaline for 3 times

Make 10 % v/v suspension with isosaline.

Hypotonicity induced haemolysis assay

Mix 1 ml of 0.15 M phosphate buffer (pH 7.4) along with 2 ml of hyposaline solution and 0.5 ml of GRBC suspension and 0.5 ml of (5-25 µg/ml) test (mesalamine). Mix 1 ml of 0.15 M phosphate buffer (pH 7.4) along with 2 ml of hyposaline solution and 0.5 ml of GRBC suspension and 0.5 ml of (5-25 µg/ml) standard (diclofenac) incubate at 37℃ for 30 min centrifuge and estimate haemoglobin content in supernatant at 560 nm. Percentage of GRBC membrane stabilisation and invitro anti-inflammatory activity was calculated.

RESULTS AND DISCUSSION

Mesalamine was evaluated for it's GRBC stabilisation activity using goat blood. The experiment involved incubating various concentrations (5,10,15,20,25 µg/ml) separately with GRBC solution and measuring the extent of haemolysis.

It was then compared with standard Diclofenac. Haemolysis was induced using hypotonic solution, which causes RBCs to burst due to osmotic pressure.

Absorbance of supernatant was measured at 560 nm, which indicated degree of haemolysis as well as the degree of protection. Lower absorbance values correspond to better stabilisation of cell membrane.

These results demonstrate a dose dependent protective effect of drug on RBC. Mesalamine effectively stabilise the cell membrane, preventing haemolysis as its concentration increases. This indicates that its potential anti-inflammatory properties and it also protect the integrity of cell membrane under stress.

Membrane stabilizing activity

Membrane stabilizing activity of mesalamine was analysed using GRBC studies and At low concentration, drug Mesalamine shows 23.49 % protection. With increasing concentrations, the stabilisation of cell membrane became more pronounced. At highest concentration, it showed 78.09 % protection.

Invitro haemolytic activity

Haemolytic activity of mesalamine was evaluated against normal goat erythrocytes .At low concentration (5 µg/ml), it reduced haemolysis to 76.50 % but with increasing concentrations, it exhibited reduction in haemolysis. At high concentration (25 µg/ml), haemolysis dropped to 21.90 %

GRBC membrane stabilisation activity

Table 1: Membrane stabilizing activity.

Concentration (µg/ml)	% protection	
	Test Mesalamine	Standard Diclofenac
5	23.49 %	20.31 %
10	31.74 %	25.70 %
15	49.84 %	26.34 %
20	61.58 %	33.33 %
25	78.09 %	45.39 %

Membrane stabilizing activity

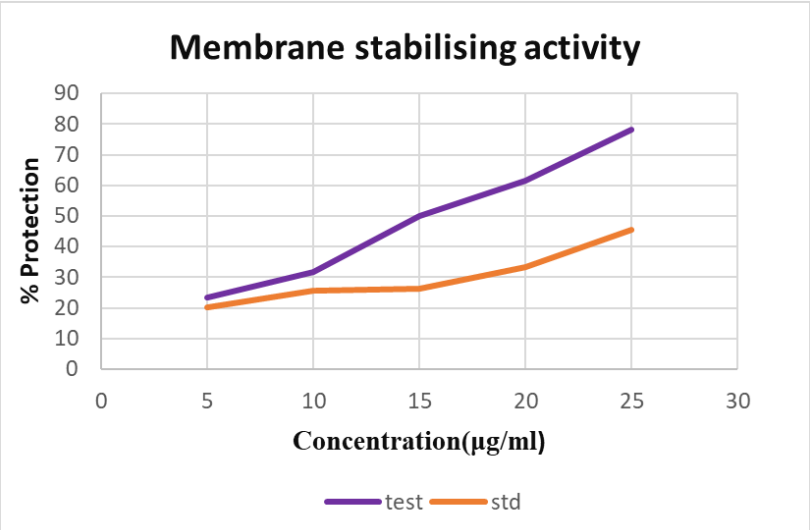

Figure 1: Bubble graph -membrane stabilizing activity.

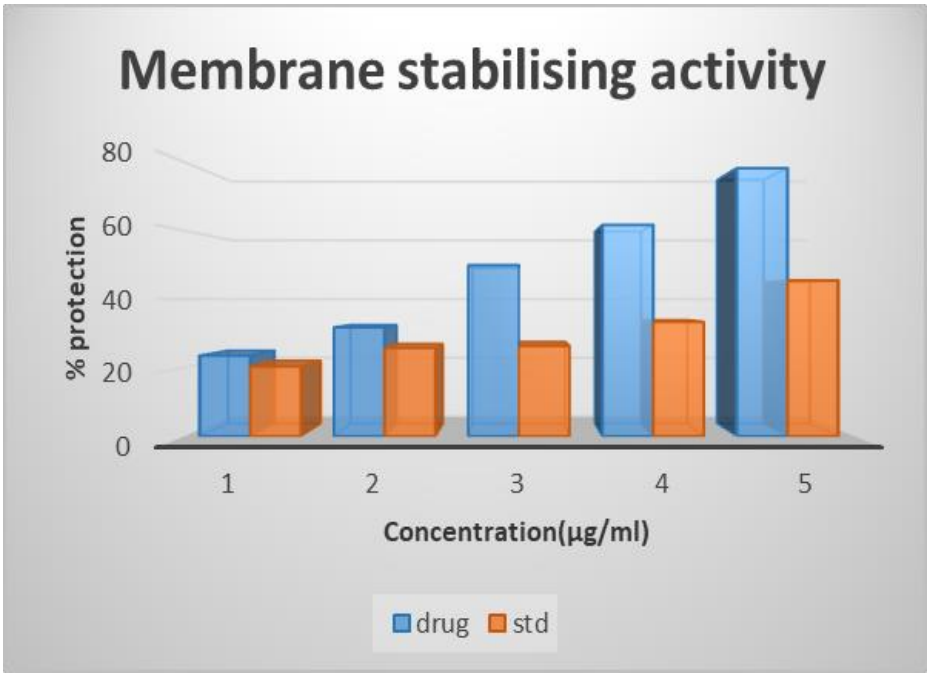

Figure 2: Bar graph – Membrane stabilising activity.

Invitro haemolytic activity

Table 2: Invitro haemolytic activity.

Concentration (µg/ml)	% haemolysis	
	Test Mesalamine	Standard Diclofenac
5	76.50 %	79.68 %
10	68.25 %	74.28 %
15	50.15 %	73.65 %
20	38.41 %	66.66 %
25	21.90 %	54.60 %

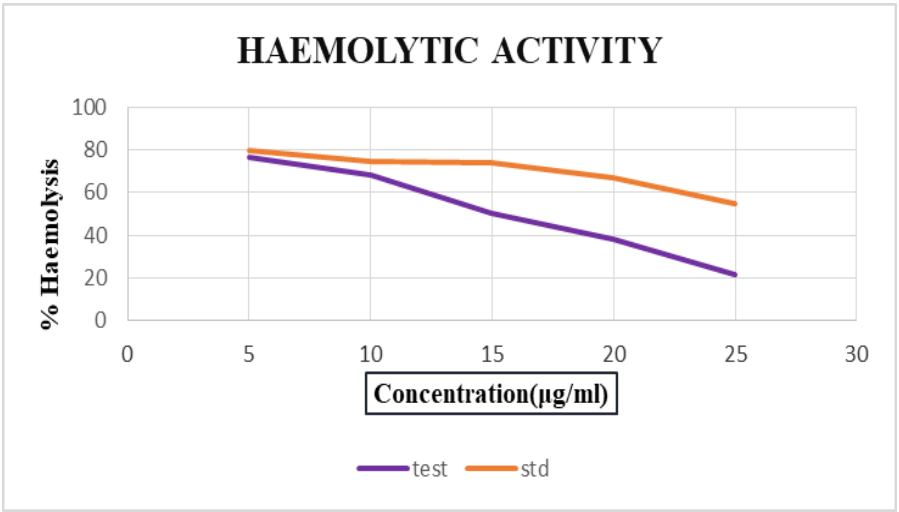

Figure 3: Bubble graph - haemolytic activity.

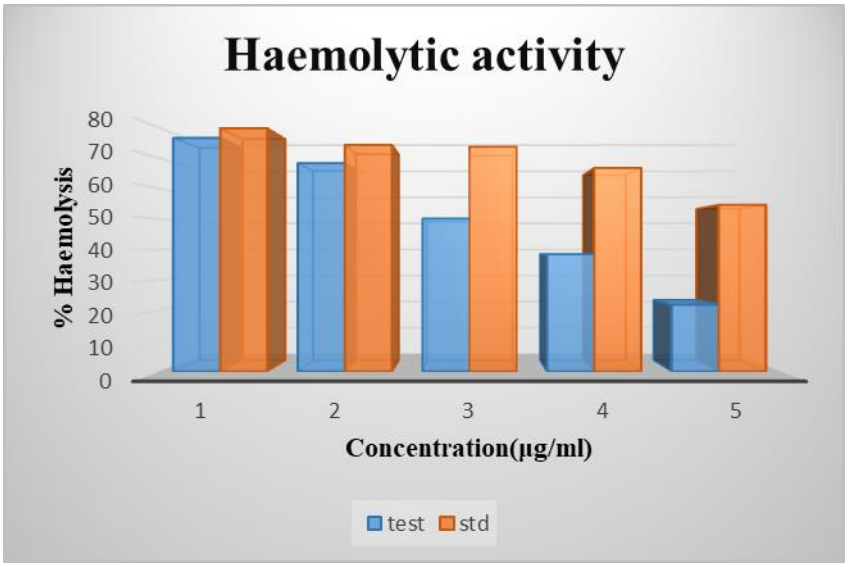

Figure 4: Bar graph – Haemolytic activity.

CONCLUSIONS

- The work aimed to explain the anti-inflammatory activity of Mesalamine using suitable screening technique-GRBC Membrane Stabilising Assay method.
- Anti-inflammatory activity confirmed by GRBC membrane stabilising activity and haemolytic activity of the mesalamine.

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