

## Effect of Glutamine on Spectrin, Clathrin and STAT-3 signaling protein in Repairing Ileum Intestine in Malnutrition Rats

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**Abstract:** Glutamine known as one of conditional essential amino acid play a role in several mechanism to support health of organism. Many researchs reported the use of glutamine to improve signalling pathways well as form and function in intestine. This research aims to study the effect of oral glutamine administration on the spectrin, clathrin expression and STAT-3 signaling protein in the repair of microvilli of intestinal epithelial mucosa damaged on malnutrition rats (*Rattus norvegicus*). Four groups of rats were used: control (group I), malnutrition (group II), normal+glutamine (group III) and malnutrition+glutamine (group IV). Group II and IV received “a karak diet” for 15 days, Group III and IV then received glutamine supplement dose of 500 mg/kg/BW for next 15 days. Then it was sacrificed to collect their intestinal ileum to be used for immunohistochemistry techniques using spectrin, clathrin and STAT-3 antibodies. Glutamine administration could increase spectrin, clathrin and STAT-3 expression on intestinal ileum on malnutrition rats. It was also showed that glutamine could repair intestinal ileum microvilli and support the improvement structure. This research could provide new insights in understanding the potency of glutamine on malnutrition rats have possibility to develope on the clinical use of glutamine in the management of malnutrition in humans.

### Introduction

Glutamine is as an immunomodulatory agent and serve to keep have function defense integrity of the intestinal mucosa to prevent mucosal atrophy, improvement permeability of the intestinal mucosal epithelium as to prevent bacterial translocation from the gut and reduce infections and also accelerates healing of damaged microvilli to be improvement in the function, structure constituent proteins and signaling pathways.

Three potential targets of glutamine administration on microvilli are increasing barrier function, cellular defense and reducing the spread of local and systemic inflammation. Research on critical illness using oral glutamine improve MAPK signaling pathway through oxidation pathways in the intestine, improve the form and function of the intestinal villi have been reported. However the mechanism of microvilli damage occurred on the fourth constituent of the protein structures in the membrane, microvillar cores, intermicrovillar and terminal web on malnutrition condition still need to be answered. This aim of this research was to explain the effect of oral glutamine administration on the spectrin, clathrin expression and STAT-3 signaling protein in the repair of microvilli of intestinal epithelial mucosa damaged on malnutrition rats (*Rattus norvegicus*).

## Materials and Methods

### Animal Preparation

Male rats (aged 12 week) were used in this study, divided into 4 groups: control (group I), malnutrition (group II), normal+glutamine (group III) and malnutrition+glutamine (group IV). Group II and IV received “a karak diet” for 15 days, Group III and IV then received glutamine supplement dose of 500 mg/kg/BW for next 15 days. On the 30th days, the animals were weighed and sacrificed. The intestine then were collected and prepared for immunohistochemistry analysis. All animal were maintained accordingly to a protocol approved by Ethical Committee of Brawijaya University, East Java, Indonesia.

### Expression of Spectrin, Clathrin and STAT-3 observations

The histo section of ileum on glas slides were dipped in xylol twice, series of vary alcohol concentration (100, 90, 80, 70, 30)%, and distilled water respectively. Then it washed in PBS pH 7.4 for 3 x 5 min, then it was soaked in 3% hydrogen peroxide (in DI water) for 5-10 min, then again washed in PBS pH 7.4 for 3 x 5 min. The section then it was soaked in 1% BSA (or 1% NGS) in PBS for 10-30 min at RT, and washed in PBS pH 7.4 for 3 x 5 min. After that the primary antibody (spectrin, clathrin and STAT-3 antibodies) was added on the preparate for 1 h at RT, and washed in PBS pH 7.4 for 3 x 5 min. The secondary antibody Anti-Rats IgG biotin labeled was added for 1 h at RT and folloowed by washing in PBS pH 7.4 for 3 x 5 min, and SA-HRP (Horseradish Peroxidase Strep-Avidin) was added for 30-60 min at RT, then it was washed in PBS pH 7.4 for 3 x 5 min. Chromogen DAB (3,3-diaminobenzidine tetrahydrochloride) were added for 10-20 min at RT, then it was washed with distilled water for 3 x 5 min. Then counterstain of Hematoxylen was applied for 5 min at RT and washed in distilled water for 3 x 5 min. The mounting with entellan was conducted and then it was observed using a light microscope with 400x [1,2]

### Data Analysis

The data expression of spectrin, clathrin and STAT-3 on ileum rats analysis was performed using Immunohistochemistry technique and were measured by axiovision program.

## Results

In malnutrition rats showed the damaged of microvilli structure of ileum histopathology. But after glutamine administration on malnutrition rats improve the intestinal villi structure of illeum also increase the expresion of Spectrin, Clathrin and STAT-3. It was confirmed by immunohistochemistry technique using primary antibodies of Spectrin, Clathrin and STAT-3.

Expression of spectrin, clathrin and STAT-3 in enterocytes on the ileum were confirmed by immunohistochemical methods. The result showed that glutamine increase the expression of spectrin, clathrin and STAT-3 on enterocytes of the ileum. This result showed that glutamine administration on malnutrition rats can improve and repair the ileum damaged (Fig. 1, 2 and 3)

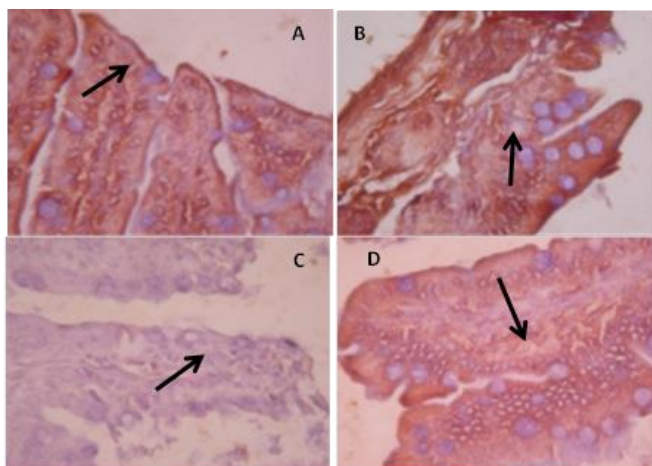
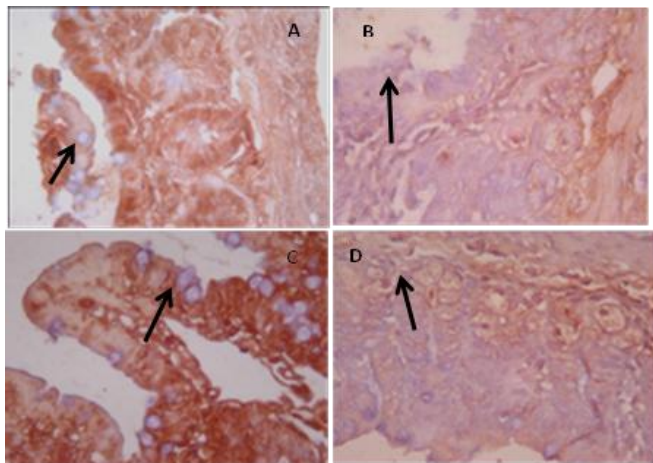


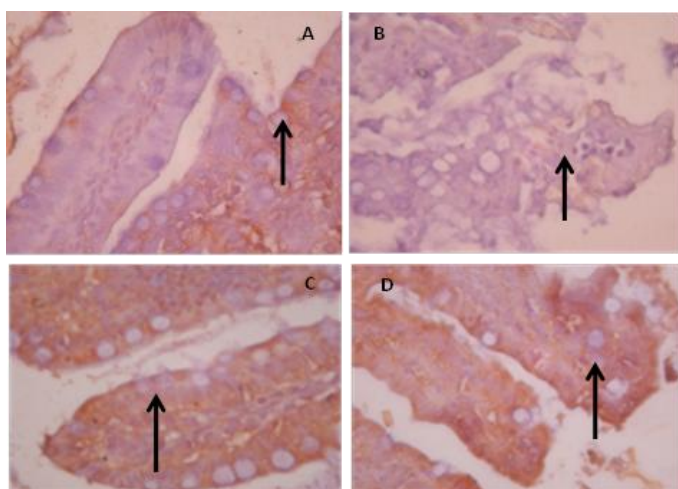
Figure 1. The spectrin expression in enterocytes ileum by immunohistochemistry technique (400x)

- A. normal rats / group I
- B. malnutrition rats/ group II
- C. glutamine administration on normal rats group/group III
- D. glutamine administration on malnutrition rats /group IV



**Figure 2. The Clathrin expression in ileal enterocytes i by immunohistochemimistry technique (400x)**

- A. normal rats / group I
- B. rats/ group II
- C. glutamine administration on normal rats group/group III
- D. glutamine administration on malnutrition rats /group IV



**Figure 3. The STAT3 expression in ileal enterocytes by immunohistochemimistry technique (400x)**

- A. normal rats / group I
- B. malnutrition rats/ group II
- C. glutamin administration on normal rats group/group III
- D. glutamine administration on malnutrition rats /group IV

### Discussion

The result showed that spectrin, clathrin and STAT-3 expression on ileal enterocytes rats increased after glutamine administration on malnutrition rats. It also showed repairing of intestinal damaged of microvilli stucture on ileum. Expression of spectrin appear more clearly in the administration glutamin on normal rats compared to malnutrition rats (Fig. 1C and Fig. 1D), but the shape and size of enterocytes were pretty much the same.

This is consistent with the theory on site mutagenesis, identified a variant of glutamine (Glu-89) and two lysine (Lys-96 and Lys-98) as an important complementary hydrophobic residues to the amino acid binding spectrin [3].

In other hand, the clathrin expression on normal rats were clearer compare to malnutrition rats which was less due to deficiency. It was also that glutamine administration on malnutrition rats could increase clathrin expression in ileal enterocytes and repaired intestinal microvilli structures damaged by malnutrition. It was indicated by the increasing of clathrin expression (Fig. 2D).

Moreover, Fig. 3 also showed the increasing of STAT3 expression on ileal enterocytes on malnutrition rats after glutamine administration during 15 days.

Glutamine showed high affinity binding clathrin, but did not cause the clathrin coat assembly. Glutamine terminal oriented on domains lining the hole on the plasma membrane by binding to arrestins and AP-2, indicates that this is the anchor that is responsible for the recruitment of receptors [3,4]

Glutamine stimulates proliferation and regulation through STAT3 signaling pathway and of Mitogen Activated Protein Kinases pathway and Jun Nuclear Kinases, also the regulation of gene expression Nuclear Transcription Factor (NTF) [5,6]

The expression of STAT3 in the ileal enterocytes increased STAT3 expression on ileal enterocytes on malnutrition rats after glutamine administration. The result means that glutamine supplementation serve to defense integrity of the intestinal mucosa, improve permeability of the intestinal mucosal epithelium as to prevent bacterial translocation from the gut and reduce infections. It is clear to explain the process repair of damaged intestinal ileum from malnutrition after glutamine administration during 15 days (Fig.3).

Effect of glutamine on the expression of spectrin in the ileum had been confirmed by our previous research. It had protein band with molecular weight of 130-250 kDa as a spectrin molecule on intestinal tissue rats. Also, the expression of clathrin and STAT 3 after glutamine administration, which had protein bands with molecular weight of 130-250 kDa and 72-95 kDa, respectively.

Glutamine is proven to have a function as repair damage to the mucosa ileum on malnutrition rats, through repairing intestinal mucosa function, protein structure and protein signaling pathways.

The result showed that the mucosal intestine damage was characterized by a collapse of the glycocalyx on the luminal surface of the lining of the intestinal absorptive cells, contain a variety of enzymes and non-enzymatic proteins including disaccharidase, peptidase, receptors, and transport proteins, that necessary for the digestion and absorption of nutrients. The main components of the glycocalyx is a carbohydrate that is embedded into the surface of the microvilli [7]

The result explained that on malnutrition condition and administration of glutamine improved structure of intestinal ileum, and proved that glutamine improve the intestinal mucosa to intestinal microvilli.

## Conclusion

The glutamine administration on malnutrition rats repaired the expression of spectrin, clathrin and STAT-3 in enterocytes in the ileum. improving structure of mucosa ileum. The potency of glutamine on malnutrition rats have possibility as a scientific basic and for the clinical used of glutamine in the management of malnutrition in humans.

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