Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

Role of *Simarouba glauca* (DC). in Restoring Intestinal Integrity during Enterotoxigenic *Escherichia coli*-Induced Diarrhea

Amrutha Lokesh Shetty[1], Veena Hedge[1], Sandesh K Gowda[2], Rajeshwara N Achur[3], Nayaka Boramuthi Thippeswamy[1,*]

[1]Department of P.G studies and research in Microbiology, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shivamogga-577451, Karnataka, India

[2]Niranthara Scientific Solutions Pvt. Ltd, Bengaluru, 560060, Karnataka, India.

[3]Department of P.G studies and research in Biochemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shivamogga-577451, Karnataka, India

(Received: 16 May 2025 Revised: 20 June 2025 Accepted: 24 July 2025)

KEYWORDS

Simarouba glauca, ETEC-induced diarrhea, disaccharidase activity, aminopeptidase N, Na^+/K^+-ATPase, intestinal enzyme modulation.

ABSTRACT:

Background: Enterotoxigenic Escherichia coli (ETEC) infection leads to gastrointestinal disturbances by impairing digestive enzyme activity, contributing to diarrheal pathology. Simarouba glauca (DC). aqueous leaf extract (SGALp) has been proposed as potential therapeutics due to its antimicrobial and anti-inflammatory properties.

Objective: To evaluate the modulatory effect of S. glauca (DC). aqueous lyophilized powder (SGALp) on activities of intestinal enzymes such as disaccharidases, aminopeptidase N, and Na^+/K^+-ATPase in ETEC-induced diarrheal BALB/c mice.

Methods: Female BALB/c mice were divided into five groups: control, ETEC-infected, ETEC + SGALp (250 mg/kg b.w), ETEC + SGALp (500 mg/kg b.w), and ETEC + Co-Trimazole (5 mg/kg b.w). Diarrhea was induced via oral ETEC H10407 inoculation (1×10^9 CFU/mL). SGALp and standard treatments were administered orally for 7 days. The disaccharidases enzyme activities in the jejunum and ileum were assayed on 1, 3, 5, and 7 days by Dahlqvist's method. The aminopeptidase N activity was measured by ELISA and the Na^+/K^+-ATPase activity was measured by spectrophotometric assay.

Results: The ETEC infection significantly reduced sucrase, maltase, aminopeptidase N, and Na^+/K^+-ATPase activities ($P < 0.001$). The SGALp treatment led to dose-dependent restoration of these enzyme activities. The SGALp at a concentration of 500 mg/kg b.w, restored enzyme activity nearly to control by day 7. Co-Trimazole showed similar but slightly less pronounced effects. The restoration of activity was statistically significant across most of the time points, especially in the higher dose group.

Conclusions: The SGALp effectively mitigated ETEC-induced reductions in key intestinal enzymes, indicating its protective and restorative potential. The aqueous extract of S. glauca demonstrated promising therapeutic efficacy in managing ETEC-associated diarrheal disorders by preserving digestive and absorptive functions of intestinal mucosa.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

Introduction

The World Health Organization (WHO) defines diarrhea as passage of three or more loose stools per day [1]. Acute diarrhea is an event that lasts less than two weeks which is triggered by bacterial, parasitic, or viral infections. It can also result from poor sanitation, food intolerance, side effects from certain medications and also from certain underlying medical conditions [2, 3]. Worldwide, diarrhea ranks as the second most common cause of death in children under the age of 5 years, representing 9% of deaths in this demographic data of about 440,000 fatalities and 50,851 deaths among children aged 5 to 9 years [4]. Diarrhea is characterized by the presence of watery stools, a rise in the frequency of bowel movements, and often includes symptoms like fever, vomiting, and imbalances in electrolytes. It ranks among the leading causes of dehydration, which can pose a serious threat to life, especially in children suffering from malnutrition or weakened immune systems [5]. Oral rehydration solution (ORS) containing salts and sugar along with zinc supplements help to restore lost fluids and electrolytes through absorption in the small intestine. Zinc supplements cut stool volume by 30% and diarrhea duration by 25%, which makes them crucial for reducing the severity [1, 6].

Even though many treatment options available, diarrhea is still a major global health concern that affects children, elderly population, and immunocompromised patients [7,8]. Enterotoxigenic *Escherichia coli* (ETEC) is a bacterial pathogen that is primarily responsible for childhood morbidity and traveler's diarrhea, especially in developing nations [9]. Colonizing ETEC produces two important enterotoxins heat-labile (LT) and heat-stable (ST) that disturb intestinal homeostasis [10]. These toxins increase the prostaglandin E2 (PGE2), cyclic GMP(cGMP) and cyclic AMP (cAMP) levels [11], which causes electrolyte imbalance, excessive fluid secretion, and enzymatic dysfunction, all these contribute towards increased severity of diarrhea [12, 13]. ORS and antibiotics are recommended conventional treatment options [14]. However, alternative therapeutic approaches are required due to the rising incidence of antimicrobial resistance [15]. Targeting digestive enzyme activity can be one of the ways to reduce the pathophysiology of ETEC diarrhea and repair intestinal function.

Among medicinal plants, *Simarouba glauca* (DC)., belonging to the Simaroubaceae family, has been traditionally used in herbal medicine across various cultures. The bark and leaves of this plant are well known for their antihelminthic, antiparasitic, antidysenteric, antipyretic, and anticancerous properties [16], [17]. Due to its historical use against dysentery, the plant's bark is commonly referred to as dysentery bark [16]. Despite its widespread traditional applications, there is limited scientific evidence supporting its role in controlling diarrhea, particularly ETEC-induced diarrhea. In spite of the traditional applications of *S. glauca* (D C) for gastrointestinal disorders, there is no comprehensive scientific study and evidence of its efficacy against ETEC-induced diarrhea. Previous research has focused on its nutritional composition and general pharmacological properties, but its mechanism of action in regulating intestinal enzyme activity and immune responses remains largely unexplored.

Additionally, while *Simarouba amara* (Aubl.) has demonstrated immunomodulatory effects in controlling ETEC pathogenesis [18], similar investigations on *S. glauca* (DC). are lacking. The absence of systematic studies on its role in modulating digestive enzymes presents a critical research gap, limiting its potential as a natural antidiarrheal agent. In view of this, the present study aims to bridge this gap by evaluating the bioactive compounds of *S. glauca* (D C)., their impact on disaccharidase, aminopeptidase N, and Na^+/K^+-ATPase activity, and their ability to restore intestinal homeostasis in ETEC-infected mice model.

Materials and Methods

Collection and preparation of *Simarouba glauca* (DC). Aqueous leaf extract lyophilised powder (SGALp)

The fresh leaves of *Simarouba. glauca* (DC). [*S. glauca* (DC).] were collected from the botanical garden of Kuvempu University, Shankaraghatta. The leaves were washed with tap water, followed by distilled water, dried in the shade for one week, and stored in air-tight polythene bags. The aqueous extract of *S. glauca* (DC). leaves was prepared according to the method described earlier [19]. Briefly, 10g of leaves were extracted with 100 ml of distilled water (1:10 w/v ratio) by boiling for 15-20 min at 121°C. After cooling, the mixture was

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

filtered through Whatman filter paper (No. 1) to obtain the aqueous extract. The extract was then concentrated by lyophilization and stored in airtight vials at 4°C.

Preparation of Bacterial Inoculum

Bacterial preparation was done according to the previous method [20]. Enterotoxigenic *E. coli* (ETEC) H10407 was grown in 2 ml Luria Broth (LB) and incubated at 37°C. Later, it was diluted 1:100 in 100 ml LB and incubated at 37°C. The ETEC H10407 growth was maintained to obtain the optical density of 0.14 at 600nm. The bacterial suspension was then centrifuged, and the pellet was resuspended in 1.6ml of sterile phosphate-buffered saline (PBS), pH 7.4, yielding a bacterial concentration of 1×10^9 CFU/ml, and 400µl aliquots were dispensed into sterile centrifuge tubes at 4°C before the inoculation in the mice model.

Animals and Group Treatment

Female BALB/c mice (22-25 g b.w) were housed at 22-25°C temperature, 50-65% humidity and 12 h light/dark cycle. They were allowed free access to food and water *ad libitum* and handled according to Institutional Animal Ethics Committee (IAEC) guidelines (NGSMIPS/IAEC/MARCH-2018/103).

Animals and Group Treatment

All the mice were acclimatized for about 72 h and were kept in sterilized cages with autoclaved bedding, drinking water and food. Before the inoculation, all the animals were pre-treated with streptomycin (5g/L) in the drinking water and fructose (6.7%), encouraging water intake up to 48 h irradiating the resident microflora [20].

The mice were divided into five groups and each group consists of five mice. Group I (control) were orally inoculated with PBS (400µl), sacrificed after 1, 3, 5, and 7 days. Group II (pathogenesis group) includes 4 subgroups. Each subgroup mice were infected with ETEC H10407 and observed for diarrheal symptoms after 1, 3, 5 and 7 days, post-infection. Group III (250 mg/kg p.o of *S.glauca* (DC). aqueous lyophilized powder (SGALp), Group IV (500 mg/kg p.o SGALp) and Group V (Co-Trimazole 5 mg/kg p.o) which are the treatment groups in which mice infected with ETEC H10407 were observed for diarrheal symptoms after 24h and treated up to seven consecutive days with one dose per day. Later, the mice feed was taken out 12 h before euthanization. After euthanization, the gastrointestinal tract was removed, washed with saline, and the jejunum and ileum segments were separated. Each segment was weighed, and its length was measured. Intestinal segments and mucosa samples were stored at –80 °C for further assays of enzyme activities.

Disaccharidases assay

The disaccharidase enzyme activities were measured by the Dahlqvist method [21, 22] and were evaluated for 1, 3, 5 and 7 days, post-infection. Groups of mice were monitored accordingly with the control group. Intestinal disaccharidase activities were assessed by measuring the amount of glucose released from maltose (EC 3.2.1. 20), and sucrose (EC 3.2.1. 48). Briefly, individual intestinal parts were opened and flushed with ice-cold PBS and the mucosa was collected by scraping with a glass slide. The mucosa was weighed and homogenised with 10 mmol PBS. Then, the homogenized sample was centrifuged at 7000*g* for 10 min at 4 °C. The supernatant was diluted at 1:50, and 1:10 with 50 mmol phosphate buffer to measure the activity of maltase, and sucrase, respectively. 10µL of diluted mucosal sample was pipetted onto a 96-well plate and an equal volume of 0.1 M sodium maleate buffer (pH 6.0) containing a solution of 0.056 M maltose, sucrose, and lactose was added to and incubated for 1 h at 37 °C. Tris-glucose oxidase was added and incubated at 37 °C for 30 min and the glucose formed during the disaccharidase hydrolysis was measured at 450 nm using glucose as standard. Disaccharidase activity was expressed as U/mg protein.

Aminopeptidase N activity

Tissue homogenates were determined for aminopeptidase N with a sandwich ELISA kit (Cusabio) according to the manufacturer's instructions. The absorbance values were read at 450nm and the aminopeptidase N activity was expressed in micrograms per mg of tissue: (µg/ml × volume of homogenate in ml)/ (weight in grams × 10) [23].

Na^+ K^+ ATPase activity

Briefly, mucosal samples of the intestinal segments were homogenised in 200 mM NaCl, 40 mM KCl, and 60 mM Tris (pH 7.4). Aliquots of 20 µL of intestinal supernatant were mixed with 20 µL of 80 mM $MgCl_2.6H_2O$, 20 µL of 20 mM EDTA, and 240 µL of distilled water. The tubes were incubated at 37° C for 5 min, 100 µl of 8 mM ATP was added, samples were mixed thoroughly and incubated at 37°C for 30 min. Following this, 200 µL of

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

5% SDS, together with 2,000 µL of Splittgerber's reagent (ammonium molybdate/sulfuric acid solution) and 9% ascorbic acid, in the ratio of 4:1 (v/v) were added, and the samples were incubated for 30 min at room temperature and allowed for the color development. The blank samples were prepared with 20 µL of distilled water instead of small intestine supernatant. The absorbance of samples was measured at 560 nm and the concentration of inorganic phosphate in the tissue was validated using a calibration curve, which was expressed in nmol/mg protein [24].

Statistical analysis

The statistical analysis was performed using Graph Pad Prism version 5.0 (GraphPad Software). Difference between the groups were interpreted with two-way ANOVA and by Bonferroni posttests. $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***) were considered as statistically significant.

Results

The physiological effects of SGALp on digestive enzyme activity in ETEC-induced diarrhea were assessed using biochemical assays. Significant changes in disaccharidase, aminopeptidase N, and Na^+/K^+-ATPase activities were observed across experimental groups, with notable differences between infected and treated mice. Treatment with SGALp demonstrated a potential protective effect, mitigating enzymatic disruptions associated with ETEC pathogenesis.

Modulation of Sucrase Activity in ETEC-Infected Jejunum

Sucrase activity in the jejunum was significantly impaired following ETEC infection. The infected group exhibited a substantial reduction in enzyme activity, with levels dropping to 8.85 ± 0.45 U/mg protein on day 1, compared to the control (38.22 ± 0.30 U/mg protein). A gradual recovery was noted over the following days, increasing to 12.08 ± 0.15 U/mg on day 3, 17.21 ± 0.31 U/mg on day 5, and 25.11 ± 0.36 U/mg on day 7, yet remaining significantly lower than the control values.

Treatment with SGALp led to notable enzyme restoration in a dose-dependent manner. In the 250 mg/kg group, sucrase activity increased from 16.26 ± 0.23 U/mg on day 1 to 17.51 ± 0.16 U/mg on day 3, 25.26 ± 0.14 U/mg on day 5, and 28.81 ± 0.38 U/mg on day 7 ($P < 0.001$). A higher dose of 500 mg/kg resulted in even greater enzyme recovery, with activity rising from 17.65 ± 0.18 U/mg on day 1 to 21.54 ± 1.68 U/mg on day 3, 26.22 ± 0.25 U/mg on day 5, and 29.71 ± 0.32 U/mg on day 7, approaching control levels ($P < 0.001$). A similar pattern was observed in the Co-Trimazole-treated group, where sucrase activity steadily improved from 12.91 ± 0.26 U/mg on day 1 to 16.80 ± 0.23 U/mg on day 3, 21.57 ± 0.19 U/mg on day 5, and 26.63 ± 0.25 U/mg on day 7 ($P < 0.001$) (Table 1).

Sucrase Activity in the Ileum During ETEC-Induced Diarrhea and Treatment Response

The sucrase activity in the ileum was significantly altered following ETEC infection, with a notable decline observed as early as day 1 post-infection. Compared to the control group, which exhibited consistent sucrase activity (33.80 ± 0.49 to 35.13 ± 0.21 U/mg protein over the study period), the ETEC-infected mice demonstrated a drastic reduction in enzyme activity, reaching a minimum of 5.79 ± 1.02 U/mg protein on day 1. However, a gradual recovery was observed over the following days, with enzyme activity increasing to 12.71 ± 0.244 on day 3 ($P < 0.001$), 16.83 ± 0.25 on day 5 ($P < 0.001$), and 22.79 ± 0.29 U/mg protein by day 7 ($P < 0.001$) (Table 1).

Treatment with SGALp showed a promising modulatory effect on sucrase activity. The lower dosage (250 mg/kg) led to a significant improvement, increasing from 15.177 ± 0.36 on day 1 to 17.21 ± 0.36 on day 3, 23.90 ± 0.51 on day 5, and 28.62 ± 0.33 U/mg protein by day 7 ($P < 0.001$). A higher dosage (500 mg/kg) resulted in a more pronounced recovery, with enzyme activity increasing from 16.92 ± 0.19 on day 1 to 20.27 ± 0.27 on day 3 ($P < 0.001$), 24.81 ± 0.50 on day 5 ($P < 0.001$), and 29.63 ± 0.26 U/mg protein on day 7 ($P < 0.001$), closely approaching control levels. Similarly, the Co-Trimazole-treated group exhibited progressive recovery, with enzyme activity improving from 11.89 ± 0.13 on day 1 to 15.93 ± 0.27 on day 3, 19.76 ± 0.20 on day 5, and reaching 25.00 ± 0.22 U/mg protein by the end of day 7 ($P < 0.001$) (Table 1).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

Table 1: Effect of ETEC Infection and SGAE Treatment on Sucrase Activity in Jejunum and Ileum

Groups	Day 1	Day 3	Day 5	Day 7
Sucrase in Jejunum				
Control	38.22±0.30	38.00±0.12	38.52±0.22	38.78±0.37
ETEC	8.85±0.45****a***	12.08±0.15****a***	17.21±0.31****a***	25.11±0.36****a***
SGALp 250	16.26±0.23****b***	17.51 ± 0.16****c***	25.26 ±0.14****d***	28.81±0.38****e***
SGALp 500	17.65±0.18****b***	21.54±1.68****c***	26.22±0.25****d***	29.71±0.32****e***
Co-Tr	12.91±0.26****b***	16.80±0.23****c***	21.57±0.19****d***	26.63±0.25
Sucrase in Ileum				
Control	33.80±0.49	33.79±0.44	34.55±0.29	35.13±0.21
ETEC	5.79±1.02****a***	12.71±0.24**** a***	16.83±0.25**** a***	22.79±0.29**** a***
SGALp 250	15.17 ± 0.36****b***	17.21 ± 0.36****c***	23.90 ±0.51****d***	28.62 ±0.33****e***
SGALp 500	16.92±0.19****b***	20.27±0.27****c***	24.81±0.50****d***	29.63±0.26****e***
Co-Tr	11.89±0.13****b***	15.93±0.27****c***	19.76±0.20****d***	25.00±0.22****e***

Data was expressed as mean ± SEM (n = 5). The statistical significance was expressed as * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$) as compared to control and infected group. 'a' indicates the comparison between pathogen infected and control group. 'b', 'c', 'd', and 'e' indicates the comparison between infected group and treated groups on day-1, day-3, day-5, and day-7, respectively.

Modulation of Maltase Activity in ETEC-Infected Small Intestine

Maltase activity was significantly impaired in both jejunum and ileum following ETEC infection, indicating enzymatic dysfunction. In the infected group, maltase activity was drastically reduced as compared to control. In the jejunum, maltase activity declined to 11.96 ± 0.31 U/mg protein on day 1, increasing to 24.77 ± 0.37 U/mg on day 3, 42.55 ± 0.42 U/mg on day 5, and 58.72 ± 0.37 U/mg on day 7, demonstrating progressive recovery but still significantly lower than control ($P < 0.001$). Similarly, in the ileum, enzyme levels dropped to 10.17 ± 0.29 U/mg protein on day 1, with a gradual recovery to 21.97 ± 0.23 U/mg on day 3, 40.18 ± 0.09 U/mg on day 5, and 49.74 ± 0.25 U/mg on day 7, yet remaining significantly lower than control ($P < 0.001$).

Treatment with SGALp significantly restored maltase activity in a dose-dependent manner. The 250 mg/kg group showed an increase from 21.47 ± 0.24 U/mg (jejunum) and 20.46 ± 0.20 U/mg (ileum) on day 1, reaching 72.53 ± 0.52 U/mg (jejunum) and 69.91 ± 0.29 U/mg (ileum) on day 7. The 500 mg/kg dose resulted in superior enzyme recovery, with maltase activity rising from 23.48 ± 0.30 U/mg (jejunum) and 21.88 ± 0.30 U/mg (ileum) on day 1, reaching 76.87 ± 0.19 U/mg (jejunum) and 75.30 ± 0.21 U/mg (ileum) on day 7, nearing control levels ($P < 0.001$).

The Co-Trimazole-treated group exhibited a steady recovery trend, with maltase activity increasing from 17.85 ± 0.22 U/mg (jejunum) and 17.52 ± 0.29 U/mg (ileum) on day 1, reaching 64.16 ± 0.34 U/mg (jejunum) and 64.08 ± 0.38 U/mg (ileum) on day 7 ($P < 0.001$) (Table 2).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

Table 2: Effect of ETEC Infection and SGAE Treatment on Maltase Activity in Jejunum and Ileum

Groups	Day 1	Day 3	Day 5	Day 7
Maltase in Jejunum				
Control	99.24±0.21	98.37±0.55	98.36±0.72	100.05±0.25
ETEC	11.96±0.31****a***	24.77±0.37****a***	42.55±0.42****a***	58.72±0.37****a***
SGALp 250	21.47±0.24****b***	36.18±0.31****c***	59.00±0.53****d***	72.53±0.52****e***
SGALp 500	23.48±0.30****b***	40.11±0.07****c***	61.61±0.24****d***	76.87±0.19****e***
Co-Tr	17.85±0.22****b***	29.85±0.18****c***	45.98±0.28****d***	64.16±0.34****e***
Maltase in Ileum				
Control	89.89±0.16	87.75±0.88	89.07±0.20	89.93±0.09
ETEC	10.17±0.29****a***	21.97±0.23****a***	40.18±0.09****a***	49.74±0.25****a***
SGALp 250	20.46±0.20****b***	36.18±0.25****c***	56.68±0.18****d***	69.91±0.29****e***
SGALp 500	21.88±0.30****b***	41.51±0.55****c***	61.56±0.46****d***	75.30± 0.21****e***
Co-Tr	17.52±0.29****b***	26.19±0.78****c***	42.97±0.42****d***	64.08±0.38****e***

Data was expressed as mean ± SEM (n = 5). The statistical significance was expressed as * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$) as compared to control and infected group. 'a' indicates the comparison between pathogen infected and control group. 'b', 'c', 'd', and 'e' indicates the comparison between infected group and treated groups on day-1, day-3, day-5, and day-7, respectively.

Effect of ETEC and SGAE Treatments on Na^+/K^+-ATPase Activity in the Jejunum and Ileum

The Na^+/K^+-ATPase activity in the jejunum was significantly reduced following ETEC infection when compared to the control group at all measured intervals ($P < 0.001$), with values ranging from 8.53 ± 0.51 to 21.79 ± 0.20, compared to control values ranging from 28.69 ± 0.70 to 29.65 ± 0.39. SGALp treatment at 250 mg/kg and 500 mg/kg significantly restored enzyme activity in the jejunum compared to the ETEC group, the activities by day 7 were 24.49 ± 0.49 and 23.78 ± 0.37, respectively ($P < 0.001$ and $P < 0.01$). However, at one intermediate time point (SGAE 500), the difference was not statistically significant ($P > 0.05$). Co-Trimazole also led to a significant improvement in enzyme activity (24.85 ± 0.46, $P < 0.001$), with no significant change observed on day 3 ($P > 0.05$) (Table 3).

Similar to the jejunum, ETEC infection significantly suppressed Na^+/K^+-ATPase activity in the ileum at all-time points ($P < 0.001$). The ETEC group showed a marked reduction in enzyme activity, with values decreasing from 6.18 ± 0.30 (nmol/mg protein) on day 1 to 14.26 ± 0.21 (nmol/mg protein) on day 7, compared to the control values ranging from 20.71 ± 0.43 to 21.41 ± 0.39 (nmol/mg protein). Treatment with SGALp at both 250 mg/kg and 500 mg/kg significantly improved Na^+/K^+-ATPase activity in the ileum across all time points ($P < 0.001$). By day 7, the enzyme activity increased to 19.63 ± 0.37 (SGALp 250) and 19.71 ± 0.18 (SGALp 500), comparable to the control. Co-Trimazole also demonstrated a protective effect, with enzyme activity reaching 18.25 ± 0.31 on day 7 ($P < 0.001$), although a non-significant difference was observed at one intermediate time point ($P > 0.05$).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

Table 3: Effect of ETEC Infection and SGAE Treatment on Na+ K+ ATPase Activity in Jejunum and Ileum

Groups	Day 1	Day 3	Day 5	Day 7
Na+ K+ ATPase in Jejunum				
Control	28.69±0.70	28.37±0.49	17.39±0.29	29.65±0.39
ETEC	8.53±0.51****a***	13.97±0.29****a***	16.83±0.25****a***	21.79±0.20****a***
SGALp250	13.35± 0.25****b***	16.48± 0.27****c***	20.24±0.18****d***	24.49±0.49****e***
SGALp 500	12.03±0.29****b***	15.16±0.21	20.17±0.50****d***	23.78±0.37****e***
Co-Tr	12.87±0.07****b***	14.12±0.19	19.21±0.4****d***	24.85±0.46****e***
Na+ K+ ATPase in Ileum				
Control	20.71±0.43	20.57±0.38	20.95±0.25	21.41±0.39
ETEC	6.18 ±0.30****a***	10.17±0.26****a***	13.21±0.21****a***	14.26±0.21****a***
SGALp 250	10.41±0.19****b***	12.82±0.30****c***	15.58±0.39****d***	19.63±0.37****e***
SGALp 500	9.23±0.20****b***	11.82±0.35****c***	15.00±0.29****d***	19.71±0.18****e***
Co-Tr	7.69±0.17****b***	12.8±0.07****c***	14.12±0.19****d***	18.25±0.31****e***

Data is expressed as mean ± SEM (n = 5). The statistical significance was expressed as * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$) as compared to control and infected group. 'a' indicates the comparison between pathogen infected and control group. 'b', 'c', 'd', and 'e' indicates the comparison between infected group and treated groups on day-1, day-3, day-5, and day-7, respectively.

Restorative Effects of SGALp on aminopeptidase N Enzyme Activities in ETEC-Infected Intestinal Tissues

ETEC infection significantly reduced aminopeptidase N activity in the jejunum at all measured time points compared to the control group ($P < 0.001$). Enzyme levels dropped from 594.50 ± 20.55 on day 1 to 950.69 ± 11.78 on day 7, whereas the control group exhibited values ranging from 1307.60 ± 211.89 to 1607.80 ± 34.33.

Treatment with SGALp at 250 mg/kg significantly improved enzyme activity at all-time points ($P < 0.001$ to $P < 0.01$), with values increasing from 823.17 ± 21.51 on day 1 to 1219.40 ± 27.77 on day 7. SGALp at 500 mg/kg also restored enzyme activity, showing a steady rise from 844.07 ± 17.61 on day 1 to 1339.40 ± 36.70 on day 7, with statistical significance observed from day 1 to day 7 ($P < 0.001$ to $P < 0.05$).

Co-Trimazole treatment resulted in a significant, though comparatively modest, improvement in enzyme activity, with values increasing from 753.95 ± 14.02 on day 1 to 1037.06 ± 40.29 on day 7 ($P < 0.001$). Overall, both SGALp doses exhibited a time-dependent restorative effect on aminopeptidase N activity in the jejunum, with SGALp 500 mg/kg showing the greatest improvement by day 7.

ETEC infection resulted in a significant and sustained reduction in aminopeptidase N activity in the ileum compared to the control group ($P < 0.001$ at all-time points). Enzyme activity in the ETEC group ranged from 554.44 ± 10.93 (U/mg) on day 1 to 911.02 ± 23.99 on day 7, whereas the control group demonstrated consistently higher values, ranging from 1407.00 ± 36.4 to 1567.60 ± 27.38.

Treatment with SGALp at 250 mg/kg significantly improved aminopeptidase N activity compared to ETEC at all-time points ($P < 0.001$), reaching 1125.40 ± 56.2

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

by day 7. Similarly, SGALp at 500 mg/kg produced a dose-dependent enhancement, with enzyme levels increasing to 1180.52 ± 28.01 by day 7 ($P < 0.001$). Co-Trimazole treatment also significantly restored aminopeptidase N activity in the ileum across all time points compared to the ETEC group ($P < 0.001$), with values increasing to 1037.06 ± 40.29 by day 7.

A similar reduction in aminopeptidase N activity was observed in the jejunum following ETEC infection, with enzyme levels ranging from 594.50 ± 20.55 to 950.69 ± 11.78, significantly lower than control values (1307.60 ± 211.89 to 1607.80 ± 34.33; $P < 0.001$ at all-time points) (Table 4).

Table 4: Effect of ETEC Infection and SGAE Treatment on Aminopeptidase N Activity in Jejunum and Ileum

Aminopeptidase N in Jejunum				
Groups	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Control	1307.60±211.89	1329.40 ±168.28	1399.80±148.5	1607.80±34.33
ETEC	594.50±20.55****a***	777.50±19.69****a***	917.00±14.01****a***	950.69±11.78****a***
SGALp 250	823.17±21.51****b***	923.40±24.49****c***	1094.80±40.76**d*	1219.40±27.77***e*
SGALp 500	844.07±17.61****b***	977.12±11.04***c*	1186.22±45.68	1339.40±36.70**e*
Co-Tr	753.95±14.02****b***	804.14±17.29****c***	930.71±25.24****d***	1037.06±40.29****e***
Aminopeptidase N in Ileum				
Control	1407.00± 36.4	1491.00±41.8	1496.80±34.8	1567.6±27.38
ETEC	554.44±10.93****a***	594.50±20.55****a***	725.44±20.20****a***	911.02±23.99****a***
SGALp 250	663.35±5.03**b*	813.17± 27.16****c***	881.24±29.59****d***	1125.40±56.2****e***
SGALp 500	732.66±16.31****b***	857.91±20.18****c***	977.12±22.96****d***	1180.518±28.01****e***
Co-Tr	708.56±23.03***b*	775.94±15.49****c***	930.71±25.24****d***	1037.06±40.29**e*

Data was expressed as mean ± SEM (n = 5). The statistical significance was expressed as * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$) as compared to control and infected group. 'a' indicates the comparison between pathogen infected and control group. 'b', 'c', 'd', and 'e' indicates the comparison between infected group and treated groups on day-1, day-3, day-5, and day-7, respectively.

Discussion

The present study investigates the protective role of *S. glauca* (D C). aqueous leaf extract (SGALp) in restoring the activity of key brush border enzymes—disaccharidases and aminopeptidase N, as well as the basolateral transporter Na^+/K^+-ATPase in ETEC-infected mice. Disaccharidases, such as sucrase and maltase, break down complex sugars into simpler forms for absorption [25], while aminopeptidase N helps in protein digestion. Na^+/K^+-ATPase is critical for maintaining ion gradients across the intestinal epithelial cells, essential for proper nutrient absorption and fluid balance [26].

Enterotoxigenic *Escherichia coli* (ETEC) infection is a major contributor to diarrheal disease [27]. The pathogenesis of ETEC is primarily driven by the secretion of LT and ST enterotoxins, which elevate intracellular cAMP, cGMP and PGE levels, leading to fluid loss, epithelial barrier dysfunction, and enzymatic suppression [18]. Studies have shown that ETEC infection significantly reduces brush border enzyme activity, including disaccharidases, aminopeptidase N,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

and Na^+/K^+-ATPase, impairing nutrient digestion and electrolyte balance [28].

The central hypothesis of this study is that the bioactive compounds present in *S. glauca* (DC) aqueous leaf extract (SGALp) can mitigate the deleterious effects of enterotoxigenic ETEC induced diarrhea by modulating digestive enzyme activities and preserving intestinal function. This hypothesis was formulated based on the traditional use of *S. glauca* (D C). in treating gastrointestinal ailments and its reported pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant activities. Given that ETEC infection disrupts intestinal homeostasis primarily through the inhibition of key digestive enzymes and impairment of ion transport mechanisms, exploring a natural product with multifaceted bioactivities presents a promising therapeutic avenue. Keeping this in view, we focused on disaccharidases, aminopeptidase N, and Na^+/K^+-ATPase because they are critical for nutrient digestion, absorption, and electrolyte balance, all of which are compromised during diarrheal episodes. Thus, assessing the physiological effects of SGALp on these enzymes provides mechanistic insights into its potential to restore intestinal function and alleviate diarrhea symptoms, thereby validating its traditional use and supporting its development as an adjunct or alternative treatment for ETEC-induced diarrhea.

In this study, the physiological effects of *S. glauca* aqueous leaf extract (SGALp) were evaluated on intestinal enzyme activities in ETEC-induced diarrhea in mice. ETEC infection caused a marked reduction in digestive enzyme activities *(*$p < 0.001$), indicating the significant mucosal and functional disruption in the small intestine. Sucrase and maltase activities were reduced by > 80% ($p < 0.001$) in both jejunum and ileum during the early stages of infection. Aminopeptidase N and Na^+/K^+-ATPase levels also significantly declined up to 60–70%, confirming the impairment of nutrient digestion and electrolyte transport due to ETEC pathogenesis.

Treatment with SGALp demonstrated a dose-dependent restoration of enzymatic function. Mice treated with 500 mg/kg SGALp exhibited near-complete recovery of maltase and sucrase activity by day 7, with a 2 to 4-fold increases as compared to the infected group. Aminopeptidase N activity improved significantly by 96 to 113% in the treated mice, reflecting restored brush-border membrane function. Na^+/K^+-ATPase activity also improved substantially (71% in the jejunum and up to 87% in the ileum), indicating improved ion transport and potential rehydration at the epithelial level. These improvements were more prominent in SGALp-treated groups than in Co-Trimazole-treated group, suggesting additional therapeutic effects beyond antimicrobial activity. The control animals maintained stable enzyme activities, while infected but untreated mice showed sustained enzyme suppression throughout the experimental timeline.

The observed effects may be attributed to the rich phytochemical profile of *S. glauca* (D C)., including triterpenoids and quassinoids known for their anti-inflammatory and antioxidant properties [16, 29]. Previous findings on *S. amara*, a closely related species, demonstrated immunomodulatory activity against ETEC-induced inflammation, with reductions in cAMP, PGE2, and intestinal damage [18]. In our study, SGALp treatment appears to have similar effects, possibly by stabilizing epithelial membranes and modulating enterotoxin-triggered signaling pathways.

Moreover, the enzymatic recovery observed in the SGALp-treated groups indicates functional restoration of the intestinal mucosa, which may contribute to improved nutrient absorption and fluid balance. The preservation or upregulation of Na^+/K^+-ATPase activity, in particular, suggests enhanced ionic reabsorption and epithelial energy homeostasis, which are critical in counteracting diarrheal fluid loss [30].

Beyond enzymatic restoration, SGALp appears to modulate intestinal immunity, as evidenced by its impact on key immune markers. Previous studies on *S. amara* demonstrated immunomodulatory effects, including reduced cAMP, PGE2, nitric oxide (NO), and MPO levels, alongside enhanced IL-1β and IAP expression [18, 31]. In our study, SGALp treatment mirrored these effects, suggesting that its bioactive compounds—quassinoids and polyphenols—may stabilize epithelial membranes and regulate enterotoxin-triggered immune responses.

Disaccharidases (e.g., lactase, sucrase, maltase) and aminopeptidase N are essential brush border enzymes located on the apical membrane of intestinal epithelial cells, playing critical roles in nutrient digestion and mucosal function. In the diarrheal state, particularly those induced by enterotoxins, their expression and

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

activity are often disrupted, leading to impaired epithelial integrity and nutrient malabsorption [32].

Recent studies suggest that preservation or upregulation of these enzymes correlates with enhanced intestinal barrier function. Aminopeptidase N, in particular, may mitigate enterotoxin-induced damage by interfering with mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) signaling pathways that are pivotal in inflammation and epithelial permeability [33]. By dampening these pathways, aminopeptidase N can reduce cytokine release and tight junction disruption, thereby stabilizing the epithelial membrane and limiting fluid loss [30]. Similarly, disaccharidase expression is a marker of enterocyte differentiation and mucosal health. Loss of these enzymes during diarrhea reflects epithelial injury, while their maintenance indicates effective mucosal defense and repair [32].

Furthermore, evidence shows that the presence of functional disaccharidases and aminopeptidase N may enhance sodium and water absorption through stimulation of Na^+/K^+-ATPase activity on the basolateral membrane [34]. This enzyme is critical for driving nutrient-coupled sodium transport, and its enhancement can counteract toxin-induced electrolyte imbalances [35].

The protective effects of *S. glauca* (DC.) aqueous leaf extract (SGALp) against ETEC-induced diarrhea likely result from multiple, synergistic mechanisms. ETEC infection elevates intracellular cAMP, cGMP and PGE2, disrupting epithelial barrier function and downregulating digestive enzymes, leading to fluid loss and mucosal injury [36, 37, 38]. SGALp may counteract this via its antioxidant-rich phytocompounds, such as quassinoids and polyphenols, which suppress oxidative stress, inhibit COX-2-mediated PGE2 production, and reduce enterotoxin-induced cAMP levels [18, 39, 40].

In summary, SGALp treatment appears to reinforce epithelial integrity, restoring digestive enzyme function while mitigating the impact of enterotoxins. By stabilizing Na^+/K^+-ATPase activity, SGALp enhances ionic balance, addressing fluid loss and electrolyte disturbances. These findings underscore its therapeutic potential in facilitating intestinal recovery and barrier protection against diarrheal damage. However, while these observations suggest promising effects, further detailed studies are required to elucidate the precise molecular mechanisms underlying SGALp's action, particularly its modulation of MAPK and NF-κB signaling pathways. Additionally, limitations such as variability in bioactive compound concentration, potential dose-dependent effects, and differences in host response should be considered for future investigations. Expanding research to human clinical trials and broader in vivo models would provide stronger translational insights for therapeutic applications.

Conclusions

The findings of this study reinforce the therapeutic relevance of *Simarouba glauca* (D C). aqueous leaf extract in mitigating ETEC-induced diarrheal pathophysiology. By restoring digestive enzyme activity, preserving epithelial integrity, and enhancing ion transport mechanisms, SGALp presents a promising natural intervention for diarrheal disorders.

Acknowledgments

The authors express their gratitude to the Department of Microbiology at Kuvempu University for the support and laboratory facilities provided.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest

References

1. World Health Organization. Diarrhoeal Disease: Global Burden, Causes, and Prevention Strategies. WHO, Geneva, 2024. https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease (accessed June 2025).
2. Wenzl H.H., Diarrhea in chronic inflammatory bowel diseases. *Gastroenterol. Clin. North Am.*, 2012, 41(3), 651–675.
3. Akhondi H., Goldin J., Simonsen K.A., Bacterial diarrhea. *StatPearls*, 2025. https://www.ncbi.nlm.nih.gov/books/NBK551643/.
4. Liang D., Wang L., Liu S., Li S., Zhou X., Xiao Y., Zhong P., Chen Y., Wang C., Xu S., Su J., Luo Z., Ke C., Lai Y., Global incidence of diarrheal diseases—An update using an

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1378-1389 | ISSN:2251-6727

interpretable predictive model based on XGBoost and SHAP: A systematic analysis. *Nutrients*, 2024, 16(18), 3217.

5. Nemeth V., Pfleghaar N., Diarrhea. In *StatPearls* [Internet], StatPearls Publishing, Treasure Island (FL), 2025. https://www.ncbi.nlm.nih.gov/books/NBK448082/.
6. Lazzerini M., Wanzira H., Oral zinc for treating diarrhoea in children. *Cochrane Database Syst. Rev.*, 2016, 12(12), CD005436.
7. Barr W., Smith A., Acute diarrhea in adults. *Am. Fam. Physician*, 2014, 89(3), 180–189.
8. Khalil I.A., Anderson J.D., Bagamian K.H., Baqar S., Giersing B., Hausdorff W.P., Marshall C., Porter C.K., Walker R.I., Bourgeois A.L., Morbidity, mortality, and long-term consequences associated with diarrhoea from *Cryptosporidium* infection in children younger than 5 years: A meta-analysis study. *Lancet Glob. Health*, 2018, 6(7), e758–e768.
9. Khalil I., Anderson J.D., Bagamian K.H., Baqar S., Giersing B., Hausdorff W.P., Marshall C., Porter C.K., Walker R.I., Bourgeois A.L., Vaccine value profile for enterotoxigenic *Escherichia coli* (ETEC). *Vaccine*, 2023, 41(Suppl 2), S95–S113.
10. Sjöling A., Qadri F., Nicklasson M., Begum Y.A., Wiklund G., Svennerholm A.M., In vivo expression of the heat stable (*estA*) and heat labile (*eltB*) toxin genes of enterotoxigenic *Escherichia coli* (ETEC). *Microbes Infect.*, 2006, 8(12–13), 2797–2802.
11. Wang H., Zhong Z., Luo Y., Cox E., Devriendt B., Heat-stable enterotoxins of enterotoxigenic *Escherichia coli* and their impact on host immunity. *Toxins*, 2019, 11(1), 24.
12. Rachmilewitz D., Karmeli F., Effect of colchicine on jejunal adenylate cyclase activity, PGE2, and cAMP contents. *Eur. J. Pharmacol.*, 1980, 67(2–3), 235–239.
13. Peterson J.W., Ochoa L.G., Role of prostaglandins and cAMP in the secretory effects of cholera toxin. *Science*, 1989, 245(4920), 857–859.
14. Casburn-Jones A.C., Farthing M.J., Management of infectious diarrhoea. *Gut*, 2004, 53(2), 296–305.
15. Neupane R., Bhathena M., Das G., Long E., Beard J., Solomon H., Simon J.L., Nisar Y.B., MacLeod W.B., Hamer D.H., Antibiotic resistance trends for common bacterial aetiologies of childhood diarrhoea in low- and middle-income countries: A systematic review. *J. Glob. Health*, 2023, 13, 04060.
16. Sahana K., Hanumanthachar J.K., A review on medicinal properties of *Simarouba glauca* DC. *Int. J. Pharm. Sci.*, 2024, 2(3), 569–578.
17. Mahesh C.D., Padyanna S., Shivamanjunath M.P., Pharmacognostical and phytochemical study on *Simarouba glauca* DC. *J. Emerg. Technol. Innov. Res.*, 2022, 9(1), A714–A721.
18. Veena H., Gowda S.K., Achur R.N., Thippeswamy N.B., Molecular mechanism of *Escherichia coli* H10407 induced diarrhoea and its control through immunomodulatory action of bioactives from *Simarouba amara* (Aubl.). *J. Microbiol.*, 2021, 59(4), 435–447.
19. Ennaifer M., Bouzaiene T., Chouaibi M., Hamdi M., *Pelargonium graveolens* aqueous decoction: A new water-soluble polysaccharide and antioxidant-rich extract. *Biomed. Res. Int.*, 2018, 2018(1), 2691513.
20. Allen K.P., Randolph M.M., Fleckenstein J.M., Importance of heat-labile enterotoxin in colonization of the adult mouse small intestine by human enterotoxigenic *Escherichia coli* strains. *Infect. Immun.*, 2006, 74(2), 869–875.
21. Dahlqvist A., Assay of intestinal disaccharidases. *Scand. J. Clin. Lab. Invest.*, 1984, 44(2), 169–172.
22. Manzano M., Giron M.D., Vilchez J.D., Sevillano N., El-Azem N., Rueda R., Salto R., Lopez-Pedrosa J.M., Apple polyphenol extract improves insulin sensitivity in vitro and in vivo in animal models of insulin resistance. *Nutr. Metab.*, 2016, 13(1), 32.
23. Yang Z., Zhu M.Z., Zhang Y.B., Wen B.B., An H.M., Ou X.C., Xiong Y.F., Lin H.Y., Liu Z.H., Huang J.A., Coadministration of epigallocatechin-3-gallate (EGCG) and caffeine in low dose ameliorates obesity and

nonalcoholic fatty liver disease in obese rats. *Phytother. Res.*, 2019, 33(4), 1019–1026.
24. Sousa N.A., Barros F.C., Araújo T.S., Costa D.S., Souza L.K., Sousa F.B., Leódido A.C., Pacífico D.M., de Araújo S., Bezerra F.F., Freitas A.L., The efficacy of a sulphated polysaccharide fraction from *Hypnea musciformis* against diarrhea in rodents. *Int. J. Biol. Macromol.*, 2016, 86, 865–875.
25. Burke M., Carbohydrate intolerance and disaccharidase measurement—A mini-review. *Clin. Biochem. Rev.*, 2019, 40(4), 167–174.
26. Gal-Garber O., Mabjeesh S.J., Sklan D., Uni Z., Nutrient transport in the small intestine: Na^+, K^+-ATPase expression and activity in the small intestine of the chicken as influenced by dietary sodium. *Poult. Sci.*, 2003, 82(7), 1127–1133.
27. Fleckenstein J.M., Kuhlmann F.M., Enterotoxigenic *Escherichia coli* infections. *Curr. Infect. Dis. Rep.*, 2019, 21, 1–9.
28. Fleckenstein J.M., Sheikh A., Emerging themes in the molecular pathogenesis of enterotoxigenic *Escherichia coli*. *J. Infect. Dis.*, 2021, 224(Suppl 7), S813–S820.
29. Ashida S., Beevi N., Raj R.S., Prabhakumari C., Qualitative detection of triterpenes and quantification of betulinic acid from hexane extract of *Simarouba glauca* leaves by gas chromatography-mass spectrometry and high-performance thin layer chromatography. *Indian J. Pharm. Sci.*, 2022, 84(6).
30. Musch M.W., Clarke L.L., Mamah D., et al., T cell activation causes diarrhea by increasing intestinal permeability and inhibiting epithelial Na^+/K^+-ATPase. *J. Clin. Invest.*, 2002, 110(11), 1739–1747.
31. Navya N., Sandesh K., Achur R.N., Thippeswamy N.B., Immunomodulatory potential of *Simarouba glauca* DC., leaf and stem bark extract, in vivo. *Biol. Forum Int. J.*, 2023, 15(4), 350–357.
32. Huërou-Luron I.L., Production and gene expression of brush border disaccharidases and peptidases during development in pigs and calves. *Biol. Growing Anim.*, 2002, 1, 491–513.
33. Tang Y., Forsyth C.B., Keshavarzian A., New molecular insights into inflammatory bowel disease-induced diarrhea. *Expert Rev. Gastroenterol. Hepatol.*, 2011, 5(5), 615–625.
34. Ghishan F.K., Kiela P.R., Epithelial transport in inflammatory bowel diseases. *Inflamm. Bowel Dis.*, 2014, 20(6), 1099–1109.
35. Anbazhagan A.N., Priyamvada S., Alrefai W.A., Dudeja P.K., Pathophysiology of IBD-associated diarrhea. *Tissue Barriers*, 2018, 6(2), e1463897.
36. Qadri F., Svennerholm A.M., Faruque A.S., Sack R.B., Enterotoxigenic *Escherichia coli* in developing countries: Epidemiology, microbiology, clinical features, treatment, and prevention. *Clin. Microbiol. Rev.*, 2005, 18(3), 465–483.
37. Field M., Rao M.C., Chang E.B., Intestinal electrolyte transport and diarrheal disease. *N. Engl. J. Med.*, 1989, 321(13), 879–883.
38. Robert A., Nezamis J.E., Lancaster C., Hanchar A.J., Klepper M.S., Enteropooling assay: A test for diarrhea produced by prostaglandins. *Prostaglandins*, 1976, 11(5), 809–828.
39. Rudrapal M., Khairnar S.J., Khan J., et al., Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials, and mechanism(s) of action. *Front. Pharmacol.*, 2022, 13, 806470.
40. Abdullah N.T., Koneri R., Screening of *Simarouba glauca* for antidiabetic and antioxidant activities. *Int. J. Pharm. Sci. Res.*, 2019, 10(1), 294–302.