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(RESEARCH ARTICLE)

# The effects of L-glutamine on castor oil induced diarrhea in albino Wistar rats

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## Abstract

Diarrhea is the condition of having three or more loose or liquid stools per day or as having more stool than normal. L-glutamine is an amino acid derived from glutamic acid. Diarrhea is a leading cause of childhood morbidity and mortality in the developing Countries. World Health Organization predicts that; there will be about 5 million deaths in children younger than five years by 2025, of which 97% will be in the developing countries. This study assessed the effects of l-glutamine on castor oil induced diarrhea in albino Wistar rats. The Study was carried out on castor oil induced diarrhea, gastrointestinal motility and on isolated rabbit jejunum. The value of ($P<0.05$) was considered as statistically significant. The total diarrheal feces weight (DF) in diarrheal control (DC) was 0.000 ±0.97. A single oral administration of each dose of L-glutamine (L-glu) (200, 400 and 600 mg/kg bw) to diarrheal rats produced significant decrease in the severity of diarrhea. The inhibition rate of wet feces mass was significant ($P<0.05$): 0.00%, 100.0%, 45.8%, 54.2% and 29.2% respectively for DC2, Lop5, L-glu200, L-glu400, L-glu600.The total length covered rate (TLCR) in diarrheic control rats was 98.0 ± 2.194%. The loperamide 5 mg/kg and L-glutamine 600 mg/kg significantly ($p<0.05$) inhibited the normal propulsion at 29.00% and 14.66% respectively. The effect of the l-glutamine on isolated rabbit jejunum revealed that l-glutamine produced a relaxation effect at 3.2 ml (400 µg/ml). The initial contraction observed with Acetylcholine was antagonized by L-glutamine.

**Keywords:** L-glutamine; Diarrhea; Loperamide; Castor oil

## 1. Introduction

Diarrhea is the condition of having three or more loose or liquid stools per day or as having more stool than is normal for that person [1]. Acute diarrhea, defined as an increased frequency of defecation (three or more times per day or at least 200 g of stools per day) lasting less than 14 days, may be accompanied by nausea, vomiting, abdominal cramping, clinically significant systemic symptoms or malnutrition [2]. Diarrheal disease and its complications remains a major cause of morbidity and mortality in children, especially in developing countries [3]. The World Health Organization (WHO) estimates that over 2.2 million deaths due to diarrheal infections occur annually, especially among children under five years of age [1]. In sub-Saharan Africa, mortality caused by acute diarrhea varies from 1.9% of all deaths in Gambia to 37% in Nigeria, with most of the deaths occurring during the first year of life [4]. Glutamine, or L-glutamine, is an amino acid derived from glutamic acid [5-7]. Glutamine is the most abundant free amino acid in the body and commonly known as a nonessential amino acid. Glutamine is present in the plasma at levels around 0.6 mM and in the intracellular space at levels around 2 and 20 mM [8-10]. It also serves as a metabolic intermediate, contributing carbon and nitrogen for the synthesis of other amino acids, nucleic acids, fatty acids, and proteins [11-12]. The main glutamine functions within the cell are separated into four categories: its role in nitrogen balance, maintaining the cellular redox state, regulation of glucose metabolism and acid base homeostasis [13]. Glutamine has an important role in cell-mediated immunity and the integrity of the intestinal mucosa. Glutamine supplementation during illness increases gut barrier and lymphocyte function and preserves lean body mass [14].

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Therefore, this study assessed the antidiarrheal activities of L-glutamine in laboratory animals. It investigated the effects of L-glutamine on percentage inhibition of castor oil-induced diarrhea in albino Wistar rats, charcoal meal intestinal transit in albino Wistar rats and on isolated rabbit jejunum.

## 2. Material and methods

### 2.1. Experimental site and source of material

All experiments were conducted in the laboratories of the Department of Pharmacology and therapeutics, Faculty of Pharmacy and the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The animals were sourced from animal house of department of veterinary physiology, faculty of veterinary medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

### 2.2. Experimental animals

New Zealand rabbit (1 kg) and albino wistar rats (150-220 g) were acclimatized in the animal house facility of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The animals were maintained on standard feed and water *ad libitum*.

### 2.3. Materials and instruments

Materials and instruments such as animal feeds, saw dust, graduated tubes, Whatmann filter papers, dissecting kit, small animal cages, starvation cages, feeding bottles, weighing balances, syringes, cotton-wools and meter rule were used. They were all obtained from the animal house and laboratories of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, and Human Physiology Department, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University, Zaria, Nigeria, where the experiments were carried out.

### 2.4. Chemicals and drugs

L-glutamine (was purchased from Sun Pharmaceutical Company Mumbai, Maharashtra, india), atropine, nifedipine, histamine, adrenaline, acetylcholine, Castor oil (Bell Sons and Co. Ltd., Southport PR9 9AL, England), Loperamide, Normal saline, Tyrode solution [composed of: NaCl (136.8 mM), KCl (2.7 mM), $CaCl_2$ (1.3 mM), $NaHCO_3$ (11.9 mM), $MgCl_2$ (0.5 mM), Na2PO4 (0.45 mM), and glucose (5.5 mM) used at a temperature of 37±1 °C]

All chemicals and drugs were obtained from the laboratories of Ahmadu Bello University, Zaria, Kaduna State, Nigeria or commercially if not available and were of analytical grade.

### 2.5. Effect of L-glutamine on castor oil-induced diarrhea

The rats were fasted for 12 hours prior to the commencement of the experiment. The methods described by Nascimento, *et al*., 2000 [15] were employed during the experiments. 25 Wistar albino rats (weighing 150-220 g) were randomly placed into five (5) groups each of five (5) animals and housed in separate cages. One cage housed one animal during the experiments. Animals of group I received 2 ml/kg normal saline orally, while those in group II received Loperamide 5 mg/kg orally, while groups III, IV and V were pre-treated with L-glutamine supplements (200 mg/kg, 400 mg/kg, and 600 mg/kg respectively) orally. Animals of groups I -V received 1ml castor oil orally using the orogastric canula 30 minutes after pre-treatment with the supplements or standard drug.

Following treatment with castor oil, the animals were then placed in separate cages over clean white paper and inspected up to 4 hours (by an observer unaware of the particular treatment) for the presence of the characteristics diarrheal droppings. The absence was recorded as a protection from diarrhea and the percentage protection was calculated using the formula;

$$\text{Percentage protection} = \frac{\text{Mean defecation of control} - \text{Mean defecation of treated group}}{\text{Mean defecation of control group}} \times 100$$

### 2.6. Effect on charcoal meal test

25 Wistar albino rats (each weighing between 150-220 g) were fasted for 18 hours and divided into five groups of five animals each. Group I received 2 ml/kg normal saline orally, group III-V received *l- glutamine* supplements (200, 400 and 600 mg/kg orally) respectively. Group II received loperamide 5 mg/kg orally. After 10 minutes one ml of marker

(10% charcoal suspension in 5% gum acacia) was administered orally to each rat. The rats were then sacrificed after 1 hour and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum [16].

$$\% \text{ Gastrointestinal transit (GIT)} = \frac{\text{Movement of charcoal (cm)}}{\text{Total length of intestine (cm)}} \times 100$$

$$\text{Percentage inhibition} = \frac{\text{Vehicle GIT} - \text{Test GIT}}{\text{Vehicle GIT}} \times 100$$

Vehicle GIT = Control and Test GIT = test group

### 2.7. Effect of L-glutamine on isolated rabbit jejunum

Rabbit (1 kg) was sacrificed by blow on the head. Segments of the jejunum, about 2.0 cm long, were removed and dissected free of adhering mesentery. The intestinal contents were removed by flushing with Tyrodes solution (NaCl, 136.8; KCl, 2.7; CaCl, 1.3; $NaHCO_3$, 12.0; MgCl, 0.5; $NaPO_4$, 0.14; glucose, 5.5. millimole). The tissue was then mounted in a 50 ml organ bath containing Tyrodes solution maintained at $37^0$C and aerated with air. A load of 0.5g was applied. Equilibration period of 60 minute was allowed during which the physiological solution was changed at every 15 min. At the end of the equilibration period, the effects of Acetylcholine ($1x10^{-5}$ µg/ml) and Adrenaline ($1x10^{-5}$ µg/ml) was determined. The effects of graded doses of the L-glutamine (0.8, 1.6 and 3.2 µg/ml) were recorded by using UgoBasile Unirecorder 7050. Also the effects of dose of the L-glutamine in the presence of agonist: Acetylcholine ($1x10^{-5}$ µg/ml) was determined, and which was incubated for 3 minutes prior to the introduction of the supplement, using UgoBasile Unirecorder 7050, instruments. The contact time for each concentration was 1 min, which is followed by washing three times. The tissue was allowed a resting period of 15 min before the next addition. Responses were recorded iso-metrically using UgoBasile Unirecorder 7050.

### 2.8. Statistical analysis

The results were reported as Mean ± SEM. Statistical analysis was performed using Statistical Product and Service Solution (SPSS), version 23.0 (2015). One-way Analysis of Variance (ANOVA) followed by Dunnet's post hoc test was done to compare the effects between control and test groups. Values with $p< 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Effect on castor oil-induced diarrhea

**Table 1: The effect of L-glutamine supplement on percentage protection from castor oil-induced diarrhea in albino Wistar rats**

| Group | Treatment | Total number of wet stools [a] | Percentage protection of diarrhea (%) |
|---|---|---|---|
| 1 | Normal saline (2 ml/kg) (Control) | 4.8 | 0.000 ±0.97 |
| 2 | Loperamide (5 mg/kg) | 0* | 100.0 ±0.00 |
| 3 | L-Glutamine (200 mg/kg) | 2.6 | 45.83 ±1.08 |
| 4 | L-Glutamine (400 mg/kg) | 2.2 | 54.17 ±0.49 |
| 5 | L-Glutamine (600 mg/kg) | 3.4 | 29.17 ±0.81 |

[a] – Mean ± SEM

4 hours after castor oil administration, the total diarrheic feces weight (DF) in diarrheal control (DC) was 0.000 ±0.97. A single oral administration of each doses of L-glutamine (L-glu) (200, 400 and 600 mg/kg bw) to diarrheic rats produced significant decrease in the severity of diarrhea, reducing the defecation rate in rats (Table 1). The frequency of stool emission was respectively: 4.8, 0, 2.6, 2.2 and 3.4/hrs for DC2, Lop5, L-glu200, L-glu400, L-glu600. The inhibition rate of wet feces mass was significant ($P<0.05$): 0.00%, 100.0%, 45.8%, 54.2% and 29.2% respectively for DC2, Lop5, L-glu 200 mg/kg, L-glu 400 mg/kg and L-glu 600 mg/kg.

### 3.2. Effect of L-glutamine on charcoal transit time

**Table 2: Effect of L-glutamine on charcoal gastrointestinal transit in the small intestine of albino Wistar rats**

| Group | Treatment | Total length of intestine (cm) | Distance travelled by charcoal (cm)[a] | Percentage of distance moved (%)[a] | Percentage inhibition of transit (%)[a] |
|---|---|---|---|---|---|
| 1 | Normal saline (2 ml/kg) | 67.5 ± 2.846 | 66.3 ± 3.767 | 98.0 ± 2.194 | 0.00 |
| 2 | Loperamide (5 mg/kg) | 69.0 ± 4.013 | 47.3 ± 7.271 | 69.6 ± 11.12 | 29.00[a] |
| 3 | L-Glutamine (200 mg/kg) | 69.0 ± 3.066 | 67.3 ± 3.176 | 97.5 ± 1.555 | 0.50[ba] |
| 4 | L-Glutamine (400 mg/kg) | 70.2 ± 3.296 | 67.4 ± 3.104 | 96.2 ± 2.824 | 1.82[ca] |
| 5 | L-Glutamine (600 mg/kg) | 72.2 ± 4.152 | 59.8 ± 2.401 | 83.6 ± 4.556 | 14.66 |

[a] – Statistically significant difference ($p < 0.05$) when compared with the control (n = 5)
[b] – Statistically significant difference ($p < 0.05$) when compared with loperamide treated group (n = 5)
[c] - Statistically signicant difference ($p < 0.05$) when compared with loperamide treated group (n = 5)

The total length covered rate (TLCR) in diarrheic control rats was 98.0 ± 2.194% (Table 2). The loperamide 5 mg/kg and L-glutamine 600 mg/kg significantly ($p<0.05$) inhibited the normal propulsion at 29.00% and 14.66% respectively.

### 3.3. Effect of L-glutamine on isolated rabbit jejunum

The effect of the l-glutamine on isolated rabbit jejunum revealed that; l-glutamine produced a relaxation effect at 3.2 ml (400 µg/ml) (Figure-1). The sustained contraction observed with acetylcholine was antagonized with atropine (Figure-2). The initial contraction observed with Acetylcholine was antagonized by L-glutamine (Figure-2).

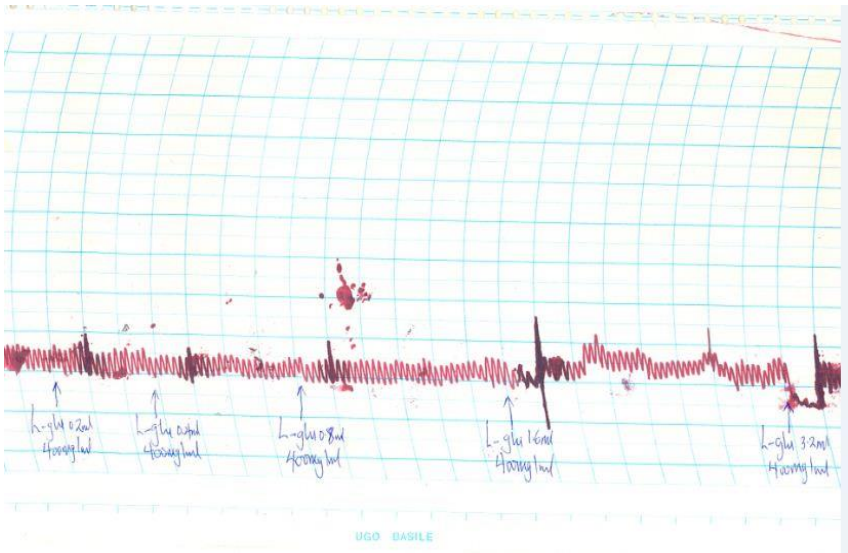

**Figure 1** The effect of graded doses of l-glutamine on isolated rabbit jejunum

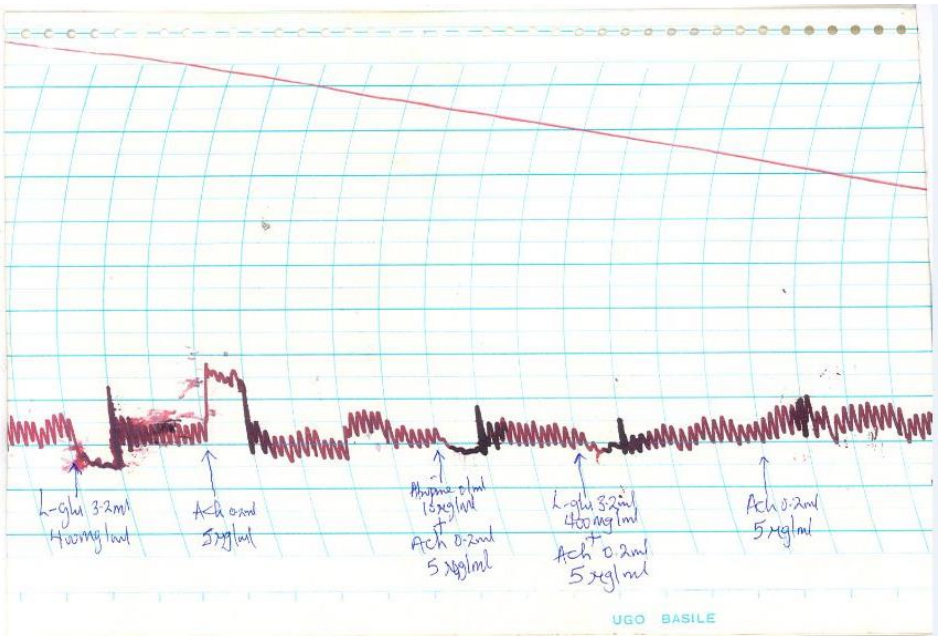


**Figure 2** The effects of doses of atropine and acetylcholine, l-glutamine and acetylcholine on isolated rabbit jejunum

## 4. Discussion

Diarrhea results from unsteady between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in intestine motility and an excess loss of fluid in the feces [17]. L-glutamine is reported to have a role in the protection of gastrointestinal mucosa [18]. The present study sought to assess the anti-diarrheal activity of l-glutamine.

L-glutamine is the most abundant free amino acids in the body. The current study shows that; the l- glutamine reduces the severity of diarrhea induced by castor oil, as indicated by the reduction of the loose stool rate and diarrhea index in wistar albino rats.

Castor oil causes diarrhea in animals due to the action of its active metabolite, ricinoleic acid, derived from hydrolysis of its triglyceride in the duodenum by pancreatic lipase. The released ricinoleic acid causes irritation, inflammation of the intestinal mucosa it also stimulates intestinal hypermotility and hypersecretion. These series of actions lead to diarrhea [19].

The L-glutamine possessed antidiarrheal activity in castor oil treated animals. The frequency and severity of castor oil induced diarrhea was inhibited in a dose dependent manner but the percentage protection of loperamide (100%) was significantly higher than the supplement ($p<0.01$). This could evident that l-glutamine effect might be mediated by an anti-secretory mechanism as loperamide by activating the intestinal Na+/K+ ATPase activity [20]. Since the l-glutamine successfully reduced severity of diarrhea induced by castor oil in this study, l-glutamine may therefore exert its anti-diarrhea effects via anti-inflammatory, anti-hypermotility or anti-hypersecretion activities. This is in line with the work of Xue *et al.*, 2008 [21] which say glutamine facilitates enteral absorption of nutrients and electrolytes in animals with experimental diarrhea.

The charcoal meal study was done to study the effect of l-glutamine on peristaltic movement. The l-glutamine and the reference drug loperamide produced anti-diarrheal effects in which they were found to decrease the distance travelled by the charcoal plug in a dose dependent manner. Both the percentage inhibition movement of loperamide (29.00%) and l-glutamine (14.66%) was significant ($p<0.05$). L-glutamine may therefore exert its effects probably through direct action on the circular and longitudinal muscles of the intestinal wall [22].

The effects of l-glutamine on isolated rabbit jejunum causes significant relaxation possibly through acting on The P2Y1 receptor, which is responsible for mediating relaxation in several areas of the Gastrointestinal tract [23]. P2Y1 receptors are coupled to a G protein (Gp) to activate phospholipase C. The latter hydrolyzes a membrane lipid to provide two second messengers, diacyl glycerol (DAG) and inositol triphosphate (IP3), which causes calcium influx from the

sarcoplasmic reticulum. Calcium activates small conductance calcium activated potassium channels sK(Ca) resulting in potassium efflux, leading to smooth muscle hyperpolarization and relaxation [24].

## 5. Conclusion

In the present study, L-glutamine supplementation showed significant antidiarrheal activity against castor oil induced diarrhea in dose dependent manner. Treatment of animals with the supplement resulted in significant decrease in the mean weight of feces compared to control. The dose of 200 mg/kg of the supplement showed 45% antidiarrheal activity whereas 400 mg/kg of the supplement showed 54 % of antidiarrheal activity. The supplement also showed a significant relaxation effect at 3.2 ml (400 μg/ml) in the isolated rabbit jejunum and decreases intestinal transit highest at 600 mg/kg. Therefore taking L-glutamine may aid in the management of diarrhea.

## Compliance with ethical standards

### *Acknowledgments*

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### *Disclosure of conflict of interest*

The authors hereby declare no conflict of interest.

### *Statement of ethical approval*

Ethical approval was obtained from Ahmadu Bello University (ABU) Committee on Animal Use and Care.

## References

[1] World Health Organization (WHO). (2012). The World Health Organization's infant feeding recommendation: Global strategy on infant and young child feeding. Genevea/New York: WHO. http://www.who.int/nutrition/topics/infantfeeding_recommendation/en/index.html (accessed on March, 14, 2017).

[2] Thielman NM and Guerrant RL. (2004). Acute Infectious Diarrhea. The England Journal of Medicine, 350(1), 38-47

[3] Cooke ML. (2010). Causes and management of diarrhea in children in a clinical setting. South African Journal of Clinical Nutrition, 23(1), Supplement S42-S46.

[4] World Health Organization (WHO)/United Nations Children Fund (UNICEF). (2004). Joint statement: clinical management of acute diarrhoea. Geneva/New York: WHO/UNICEF. http://www.who.int/child_adolescent_health/documents/who_fch_cah 04_7/en/index.html (retrieved on March, 14 2017).

[5] Gstraunthaler G, Holcomb T, Feifel E, Liu W and Spitaler N. (2001). Differential expression and acid-base regulation of glutaminase mRNAs in gluconeogenic LLC-PK(1)-FBPase(+) cells. American Journal of Renal Physiology, 278(2), 227-237.

[6] Oliveira GP, Diasi CM, Pelosi P and Patricia RM. (2010). Understanding the mechanisms of glutamine action in critically ill patients, 82(2), 417-430.

[7] Pérez-Bárcena J, Crespí C, Regueiro V, Marsé P, Raurich JM and Ibáñez J. (2010). Lack of effect of glutamine administration to boost the innate immune system response in trauma patients in the intensive care unit. Critical Care, 14(6), R233.

[8] Cetinbas F, Yelken B and Gulbas Z. (2010). Role of glutamine administration on cellular immunity after total parenteral nutrition enriched with glutamine in patients with systemic inflammatory response syndrome. Journal of critical care, 25(4), 661-e1.

[9] Martins P, Alves L, Santos Rosa M, Lemos L, Casanova P and Falcão J. (2005). Effects of L-alanyl L-glutamine dipeptide-supplemented parenteral nutrition on lymphocyte subpopulations and in prevalence of nosocomial infection in critically ill patients. Critical Care, 9(1), P364.

[10] Tao KM, Li XQ, Yang LQ, Yu WF and Lu ZJ. (2014). Glutamine supplementation in critically ill adults. Cochrane Database System Review, 9, CD010050.

[11] Heyland D, Muscedere J, Wischmeyer P.E, Cook D, Jones G, Albert M, Elke G and Berger MM. (2013). Canadian Critical Care Trials Group. A randomized trial of glutamine and antioxidants in critically ill patients. England Journal of Medicine, 368(16), 1489-97.

[12] Andrews PJ, Avenell A, Noble DW, Campbell MK, Croal BL, Simpson WG, Vale LD, Battison CG and Jenkinson DJ. (2011). Randomised trial of glutamine, selenium, or both, to supplement parenteral nutrition for critically ill patients. BMJ, 342, D1542.

[13] Novak F, Heyland DK, Avenell A, Drover JW and Su X. (2002). Glutamine supplementation in serious illness: a systematic review of the evidence. Critical care medicine, 30(9), 2022-2029.

[14] Chen QH, Yang Y, Xie JF and Liu AR. (2014). The effect of glutamine therapy on outcomes in critically ill patients: a meta-analysis of randomized controlled trials. Critical Care, 18(1), R8.

[15] Nascimento GGF, Locatelli J, Paulo CF and Silva G. (2000). The antimicrobial activity of plant extract and phytochemical on antibiotics resistant. Brazilian Journal of Microbiology, 31, 247-256.

[16] Diurno MU, Izzo AA, Mazzoni B, Bologgnese A and Capaso F. (1996). Anti-diarrheal activity of new thiazolidinones related to loperamide. Journal of Pharmacy and Pharmacology, 45, 1054 - 1059.

[17] Anup M, Saikat D and Subhash CM. (2007). *In vivo* evaluation of antidiarrhoeal activity of the seed of *Swietenia macrophylla* King (Meliaceae). Tropical Journal of Pharmaceutical Research, 6(2), 711 – 716.

[18] Newsholme P, Procopio J, Lima MM, Pithon-Curi TC and Curi R. (2003). Glutamine and glutamate - their central role in cell metabolism and function. Cell Biochemical Function, 21, 1-9.

[19] Ammon PJ, Thomas and Philips S. (1974). Effects of oleic and recinoleic acids net jejunal water and electrolyte movement. Journal of Clinical Investigation, 53, 374-379.

[20] Amos S, Okwusaba FK, Gamaniel K, Akah P and Wembebe, C. (1998). Inhibitory effects of the aqueous extracts of *Pavetta crassipes* leaves on gastrointestinal and uterine smooth muscle preparation isolated from rabbit, guinea-pig and rats. Journal of Ethnopharmacology, 60, 209-212.

[21] Xue H, Sawyer MB, Field CJ, Dieleman LA, Murray D and Baracos VE. (2008). Bolus oral glutamine protects rats against CPT -II-induced diarrhea and differentially activates cytoprotective mechanisms in host intestine but not tumor. Journal of Nutrition, 138, 740-746.

[22] Ooms LA, Degryse AD and Janssen PA. (1984). Mechanisms of action of loperamide. Journal of Gastroenterology Supplement, 96,145-55.

[23] Jiménez M, Clave P and Accarino A. (2014). Purinergic neuromuscular transmission in the gastrointestinal tract, functional basis for future clinical and pharmacological studies. British Journal of Pharmacology, 171, 4360-75.

[24] Co€effie M, Letellier RM and Dechelotte P. (2010). Potential for Amino Acids Supplementation during Inflammatory Bowel Diseases, 16(3), 518-524.

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