International Journal of

Biological Chemistry

ISSN 1819-155X

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Biological Chemistry

ISSN 1819-155X
DOI: 10.3923/ijbc.2020.8.13

Research Article
Effect of Vitamin C Alone and in Combination with Loperamide on Castor Oil-induced Diarrhea

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Abstract

Background and Objective: Diarrhea is a killer disease especially amongst children. Oxidative stress is one of the causes of diarrhea. This study investigated the effect of an antioxidant (vitamin C) when administered alone and in combination with loperamide, an antidiarrheal drug, in castor oil-induced diarrhea. **Materials and Methods:** Adult albino rats were administered 25-150 mg kg^{-1} b.wt. vitamin C alone and in combination with 3 mg kg^{-1} b.wt. loperamide. The antidiarrheal activity was assessed using standard procedures. The concentration of malondialdehyde (MDA) and Na^{+}-K^{+} ATPase activity in small intestine homogenates were also evaluated. Antioxidant enzymes activity and MDA concentration in rat small intestinal homogenates after 5 days co-administration of 50, 100 and 200 mg kg^{-1} b.wt. of vitamin C with 3 mg kg^{-1} b.wt. loperamide was evaluated and compared with loperamide alone and normal rats. **Results:** There was a significant decrease ($p<0.05$) in the number of wet stools at 100 and 150 mg kg^{-1} b.wt. Vitamin C alone and in combination with loperamide. The weight and volume of intestinal fluids as well as the small intestinal MDA concentration of rats administered 50 mg kg^{-1} b.wt. vitamin C and loperamide significantly decreased when compared to other treatments. Administration of 50-200 mg kg^{-1} b.wt. vitamin C and loperamide for 5 days significantly decreased catalase and glutathione peroxidase activities and the concentration of MDA when compared with loperamide alone. **Conclusion:** These findings indicate that vitamin C when combined with loperamide has better antidiarrheal activity and reverses oxidative stress caused by prolonged usage of loperamide.

Key words: Oxidative stress, vitamin C, antioxidant, diarrhea, loperamide, reactive oxygen species and lipid peroxidation

Citation: M.U. Ahmed, M.S. Hauwa'u, A.A. Modibbo, F.Z. Ahmed, G. Marcus and U. Emmanuel, 2020. Effect of vitamin C alone and in combination with loperamide on castor oil-induced diarrhea. Int. J. Biol. Chem., 14: 8-13.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diarrhea is a common illness in developing countries. It is an alteration in the normal bowel movement[1]. Oxidative stress has been implicated in the pathophysiology of gastrointestinal disturbances such as diarrhea[2,3]. A major cause of diarrhea is microbial or parasitic infection. Viral, bacterial or fungal infection causes the release of Reactive Oxygen Species (ROS) by phagocytes[4]. Rotavirus, for example, induces chloride secretion through the generation of reactive oxygen species and a decrease in reduced glutathione and glutathione ratio (GSH/GSSG)[5]. Intestinal hypersecretion that occurs in diarrhea is usually accompanied by hydrogen peroxide (H_2O_2) generation[3]. Hydrogen peroxide itself is not toxic but may lead to the formation of toxic hydroxyl radicals which can cause lipid peroxidation and can induce depletion of glutathione. Lipid peroxidation results in an alteration in membrane fluidity, loss of membrane integrity and disruption in ion transport, resulting to diarrhea[6]. Also, oxidants such as H_2O_2 induces anion secretion[7,8]. There is increased oxidative stress and oxidant capacity in Inflammatory Bowel Disease (IBD), a disease characterized by diarrhea[9]. A recent study reported that prolonged usage of loperamide, a widely used[8] antidiarrheal drug, causes a jejunal oxidative stress state in the jejunum[10].

Despite evidence linking the generation of reactive oxygen species and oxidative stress to diarrhea, there is little information on the use of known antioxidants in the treatment and management of diarrhea. Vitamin C (also known as ascorbic acid) is a water-soluble micronutrient with antioxidant properties[11]. It is a potent reducing agent and scavenger of oxidizing free radicals and harmful oxygen-derived species such as singlet oxygen, hydrogen peroxide and hydroxyl radicals[12,13]. It is involved in the first line of antioxidant defense, in protecting proteins and lipid membranes from oxidative damage[11]. This study aims at evaluating the effect of vitamin C alone and in combination with loperamide in the treatment of castor oil-induced diarrhea.

MATERIALS AND METHODS

Study area: This research project was conducted from November, 2019 to January, 2020 at the research laboratory, Biochemistry Department, Adamawa State University, Mubi, Adamawa, Nigeria.

Experimental animals: Adult albino rats of both sexes weighing between 120-150 g were used. The rats were housed in a well-ventilated aluminum cage. The rats were fed with standard laboratory diet. They were handled according to the guidelines for protecting and handling laboratory animals by the International Council for Laboratory Animal Science (ICLAS).

Antidiarrheal activity: Adult rats were randomly divided into eight groups after induction of diarrhea by the method described by Awouters *et al.*[14]. Animals in each group were treated with normal saline (diarrheal control), 3 mg kg^{-1} b. wt. loperamide, 25-150 mg kg^{-1} b.wt. vitamin C alone and 3 mg kg^{-1} b. wt. loperamide plus 25-150 mg kg^{-1} b.wt. vitamin C respectively. Animals were placed in separate cages lined with foil paper. The number of diarrheal stool was monitored for 4 h. The method described by Robert *et al.*[15] was adopted for the gastrointestinal transit time. The various treatment was administered an hour after inducing diarrhea. Thirty minutes after, 1 mL of a charcoal meal (10% suspension in 5% gum acacia) was administered to the rats. The rats were sacrificed by ether anesthesia and the small intestine was carefully separated. Small intestinal transit was calculated as percentage distance traveled by charcoal meal to the total length of the intestine. The intestinal fluid accumulation test was done as described by Mascolo *et al.*[16]. Briefly, an hour after castor oil administration, the various treatments were given and rats were anesthetized with ether after an hour. The small intestine was carefully removed and weighed. The intestinal content was milked into a graduated measuring cylinder and the volume was taken. The difference between the full and empty intestine was calculated. The small intestine homogenate was prepared in 0.25 M sucrose (1:4). The concentration of malondialdehyde and Na^+-K^+ ATPase activity of the homogenate was determined as described by previous research[17,18].

Effect of vitamin C administration on prolonged usage of loperamide: The albino rats were randomly divided into five groups of five rats each. Group, I served as the control and received normal saline. Group II received 3 mg kg^{-1} b.wt. loperamide while groups III-V received 3 mg kg^{-1} b.wt. loperamide and 50, 100 and 200 mg kg^{-1} b.wt. vitamin C respectively. The treatment was administered once a day for five days. On the 6th day, animals were sacrificed by ether anesthesia. The small intestine was removed and the content emptied. The small intestine was homogenized in 0.25 M

sucrose in the ratio 1: 4. The small intestinal homogenate was used to determine the malondialdehyde concentration using the thiobarbituric acid[17], catalase activity[19], superoxide dismutase activity[20] and glutathione peroxidase activity[21].

Statistical analysis: Statistical analysis was done using SPSS version 24.0 software. Data are expressed as mean±standard error means of five determinations. Data were statistically analyzed using one-way analysis of variance and Duncan multiple range test. The $p<0.05$ was considered significant.

RESULTS

The result of the effect of co-administration of vitamin C with loperamide on antidiarrheal parameters is presented in Table 1. Administration of 100 and 150 mg kg^{-1} b.wt. vitamin C alone and in combination with 3 mg kg^{-1} b.wt. loperamide significantly decreased ($p<0.05$) the number of diarrheal stool from 3.00±0.00 to 0.00±0.00. Only 3 mg kg^{-1} b.wt. loperamide significantly decrease ($p<0.05$) the small intestinal transit from 61.66±0.07 to 56.22±2.82. The small intestinal transit of vitamin C alone (at all doses tested for) and its combination with loperamide significantly increased ranging from 70.52±5.24 to 98.20±2.55. All the treatments significantly decreased the weight and volume of the intestinal fluid when compared with loperamide and control groups. Addition of 50, 100 and 150 mg kg^{-1} b.wt. vitamin C to loperamide significantly decreased ($p<0.05$) the weight and volume of the intestinal fluid when compared to vitamin C alone groups. The most significant reduction was at 50 mg kg^{-1} b.wt. 0.78±0.14 and 0.77±0.20, respectively.

All the treatment significantly decreased the concentration of MDA from 1.60±0.08 to a range of between 0.07±0.01 to 0.73±0.30 as shown in Fig. 1. The lowest reduction was at 50 mg kg^{-1} b.wt. (0.07±0.01). The concentration of MDA significantly increases at 100 to 0.27±0.09 mg kg^{-1} b.wt.

Table 2 shows the effect of co-administration of vitamin C at different doses with 3 mg kg^{-1} b.wt. loperamide on small intestinal Na^+-K^+ ATPase activity. The activity of Na^+-K^+ ATPase of the diarrheal control group significantly decreased to 5.34±1.13 when compared to the normal control (8.48±0.05). Administration of loperamide alone and the co-administration of loperamide and vitamin C significantly increased Na^+-K^+ ATPase activity when compared with the diarrheal group. However, the increase was not comparable to normal control.

The small intestinal MDA concentration of rats administered loperamide alone significantly increased to 22.81±0.48 when compared to the normal control (18.53±0.64) as shown in Table 3. Co-administration of loperamide with 200 mg kg^{-1} b.wt. Vitamin C significantly decreased MDA concentration to 15.07±1.27.

The effect of co-administration of vitamin C and loperamide on antioxidant enzymes is shown in Table 4. Administration of loperamide alone significantly increased the activity of glutathione peroxidase, catalase and superoxide

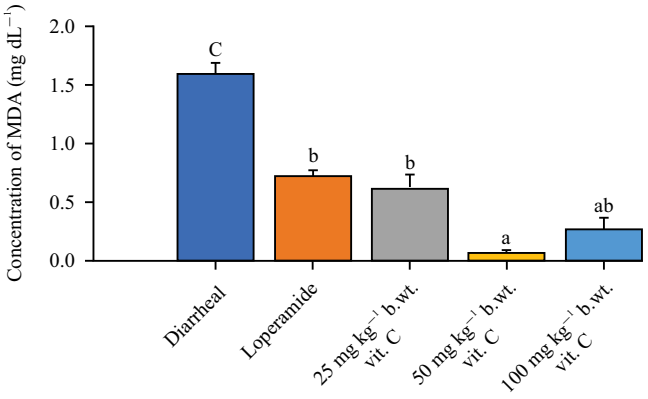

Fig. 1: Concentration of malondialdehyde (MDA) in small intestine homogenates of diarrheal rats treated with vitamin C

Values are mean of five replicates±SEM, bars with different superscript are significantly different ($p<0.05$)

Table 1: Effect of administration of vitamin C alone and in combination with loperamide on antidiarrheal parameters

Groups	No. of wet stool	Small intestinal transit (%)	Weight of intestinal fluid (mg)	Volume of intestinal fluid (mL)
Control	3.00±0.00^{d}	61.66±0.07^{b}	3.96±0.17^{f}	3.12±0.15^{e}
3 mg kg^{-1} b.wt. loperamide	0.67±0.33^{b}	56.22±2.82^{a}	1.98±0.35^{e}	2.01±0.13^{d}
50 mg kg^{-1} b.wt. vit. C	0.67±0.33^{b}	82.30±5.04^{c}	1.16±0.17^{c}	1.27±0.13^{c}
100 mg kg^{-1} b.wt. vit. C	0.00±0.00^{a}	87.47±7.36^{c}	1.73±0.54^{d}	1.55±0.48^{c}
150 mg kg^{-1} b.wt. vit. C	0.33±0.33^{a}	75.85±5.11^{c}	1.84±0.31^{d}	1.87±0.29^{cd}
50 mg kg^{-1} b.wt. vit. C + loperamide	1.00±0.00^{c}	70.42±5.24^{c}	0.78±0.14^{a}	0.77±0.20^{a}
100 mg kg^{-1} b.wt. vit. C + loperamide	0.00±0.00^{a}	76.29±2.56^{c}	0.98±0.06^{b}	1.08±0.07^{b}
150 mg kg^{-1} b.wt. vit. C + loperamide	0.00±0.00^{a}	98.20±2.55^{d}	0.88±2.55^{b}	1.20±0.12^{bc}

Values are mean of five replicates±SEM, values with different superscript down the column are significantly different ($p<0.05$)

Table 2: Effect of co-administration of vitamin C and loperamide on Na^+-K^+ ATPase activity

Groups	Na^+-K^+ ATPase activity (U L^{-1}) $\times 10^{-5}$
Control	8.48±0.05[b]
Diarrheal	5.34±1.13[a]
3 mg kg^{-1} b.wt. loperamide	6.70±0.58[a]
25 mg kg^{-1} b.wt. vit. C + loperamide	6.06±0.93[a]
50 mg kg^{-1} b.wt. vit. C + loperamide	6.70±1.10[a]
100 mg kg^{-1} b.wt. vit. C + loperamide	6.18±0.42[a]

Values are mean of five replicates±SEM, values with different superscript down the column are significantly different ($p<0.05$)

Table 3: Effect of co-administration of vitamin C and loperamide for five days on lipid peroxidation

Groups	MDA (mg dL^{-1})
Control	18.53±0.64[ab]
3 mg kg^{-1} b.wt. loperamide	22.81±0.48[b]
50 mg kg^{-1} b.wt. vit. C + loperamide	18.51±0.28[ab]
100 mg kg^{-1} b.wt. vit. C + loperamide	18.50±0.27[ab]
200 mg kg^{-1} b.wt. vit. C + loperamide	15.07±1.27[a]

Values are mean of five replicates±SEM, values with different superscript down the column are significantly different ($p<0.05$), MDA: Malondialdehyde

Table 4: Effect of co-administration of vitamin C and loperamide for five days on the activity of antioxidant enzymes

Groups	GP× (U L^{-1})	CAT (U L^{-1})	SOD (U L^{-1})
Control	3.07±0.06[a]	0.27±0.03[a]	1.17±0.33[a]
3 mg kg^{-1} b.wt. loperamide	3.93±0.19[b]	1.28±0.17[c]	2.00±0.58[b]
50 mg kg^{-1} b.wt. vit. C + loperamide	3.58±0.17[a]	0.50±0.25[b]	1.30±0.01[b]
100 mg kg^{-1} b.wt. vit. C + loperamide	3.51±0.25[a]	0.50±0.03[b]	1.43±0.05[b]
150 mg kg^{-1} b.wt. vit. C + loperamide	3.59±0.28[a]	0.51±0.19[b]	2.00±0.50[b]

Values are mean of five replicates±SEM, values with different superscript down the column are significantly different ($p<0.05$), GPx: Glutathione peroxidase, CAT: Catalase, SOD: Superoxide dismutase

dismutase from 3.07±0.06 to 3.93±0.19, 0.27±0.03 to 1.28±0.17 and 1.17±0.33 to 2.00±0.58, respectively. Co-administration of 50, 100 and 200 mg kg^{-1} b.wt. vitamin C significantly decreased ($p<0.05$) the activity of glutathione peroxidase and catalase when compared to the groups administered loperamide alone. There was no significant difference in the small intestine superoxide dismutase activity of rats administered loperamide alone and loperamide plus vitamin C.

DISCUSSION

The significant reduction in the number of wet stool by vitamin C alone and in combination with loperamide may be due to the antisecretory and/or antioxidant potential of vitamin C. The active component of castor oil, ricinoleic acid, causes intestinal mucosa inflammation which stimulates the production of several mediators such as prostaglandins. PGE_2 elicit net secretion of fluid, chloride and bicarbonate ions and inhibit sodium absorption to cause hypersecretion[22,23]. Prostaglandins alters the motility of the gastrointestinal tract[24]. It also inhibits intestinal Na^+-K^+ ATPase[25]. This leads to diarrhea. Inhibitors of prostaglandin synthesis have antisecretory properties by decreasing secretion of fluid into the lumen[26]. Antioxidants such as vitamin C inhibit prostaglandin synthesis by inhibition of peroxidation of phospholipids[27].

The non-significant reduction in the small intestine transit by vitamin C suggests that it has no anti-motility activity. This suggests that the antidiarrheal activity of vitamin C is due to its antioxidant activity rather than its ability to inhibit prostaglandin synthesis since prostaglandin can stimulate gastrointestinal motility. Umokoro and Ashorobi [28] also reported that vitamin C has no antimotility effect. The ability of vitamin C to prevent or reduce intraluminal fluid accumulation is by maintaining small intestine membrane integrity via its antioxidant property. Castor oil has been reported to cause an increase in the generation of malondialdehyde, an index of lipid peroxidation and this suggests a possible mechanism of tissue alteration due to reactive oxygen derivatives[3,29,30]. Tissue alteration can lead to destruction in cellular functions and disruptions in ion transport resulting in diarrhea[6]. Thus, by scavenging for Reactive Oxygen Species (ROS), tissue integrity in the gastrointestinal tract is maintained to prevent diarrhea. The better anti-enter polling activity at a lower dose of vitamin C and loperamide may be due to the pro-oxidant activity of vitamin C. Castor oil-induced diarrhea produces free iron and H_2O_2[31]. In the presence of free metal ions, excess ascorbic acid can promote and initiate a free radical reaction through the Fenton reaction. It can reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions which can in turn reduce H_2O_2 to hydroxyl radical (a potent free radical)[11]. This pro-oxidant activity of vitamin C is also evidenced by the higher MDA

concentration at 100 mg kg^{-1} b.wt. in comparison with 50 mg kg^{-1} b.wt. indicating more lipid peroxidation due to ROS generation.

Na^+-K^+ ATPase plays an important role in the maintenance of water and electrolytes and it is inhibited in all types of diarrhea[32-34]. Neither loperamide nor its combination with vitamin C restored its activity to normal indicating that these treatments do not have any effect on the activity of the enzyme.

The significant increase in MDA concentration in the loperamide group indicates lipid peroxidation and an increase in the activities of the antioxidant enzymes in the loperamide group indicates oxidative stress. Oxidative stress is characterized by an imbalance between reactive oxygen species production and the level of the antioxidant defense system in favor of reactive oxygen species generation[2]. Excessive reactive oxygen species induce lipid peroxidation which is evidenced by the increased MDA concentration in the loperamide group. Prolonged usage of loperamide s leads to iron and hydrogen peroxide overload[35]. Iron and H_2O_2 are the Fenton reagent that generates hydroxyl radicals that can attack biological materials such as lipids resulting in lipid peroxidation[10,36,37]. Vitamin C at all tested doses reversed all loperamide induced oxidative stress to near control levels. This may be due to the antioxidant activity of vitamin C to chelate free iron and scavenge effectively for hydrogen peroxide[38]. Catalase and glutathione peroxidase converts hydrogen peroxide to H_2O and O_2[39]. Usually, the antioxidant defense systems including the antioxidant enzymes are induced in response to increased ROS production[40]. Thus, the decrease in their activities upon administration of vitamin C indicates a decreased concentration of hydrogen peroxide due to the effective scavenging of hydrogen peroxide by vitamin C.

CONCLUSION

Vitamin C has anti-diarrheal activity via its antioxidant property. Its co-administration with loperamide is more efficient than it alone or loperamide alone. It also prevents oxidative stress caused by prolonged administration of loperamide. Thus, antioxidants play a significant role in the treatment of diarrhea.

SIGNIFICANCE STATEMENT

This study discovered that vitamin C has anti-diarrheal activity and that its co-administration with loperamide will be beneficial in the treatment of diarrhea. This study will help the researcher to justify the use of antioxidants such as vitamin C in the treatment of diarrhea which many researchers have not explored. Thus, a new theory on the use of loperamide with vitamin C or other antioxidants may be arrived at.

ACKNOWLEDGMENT

The authors wish to acknowledge and thank Mr. Noseh Janyo Dahiru for his assistance during the laboratory work.

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