Akinleye Stephen Akinrinde* and Halimot Olawalarami Hameed

Glycine and L-Arginine supplementation ameliorates gastro-duodenal toxicity in a rat model of NSAID (Diclofenac)-gastroenteropathy via inhibition of oxidative stress

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Abstract

Objectives: This study examined the possible protective roles of exogenous glycine (Gly) and L-Arginine (L-Arg) against Diclofenac (DIC)-induced gastro-duodenal damage in rats.

Methods: Rats were divided into Group A (control), Group B (DIC group) and Groups C–F which were pre-treated for five days with Gly1 (250 mg/kg), Gly2 (500 mg/kg), L-Arg1 (200 mg/kg) and L-Arg2 (400 mg/kg), respectively, before co-treatment with DIC for another three days. Hematological, biochemical and histopathological analyses were then carried out.

Results: DIC produced significant ($p<0.05$) reduction in PCV (13.82%), Hb (46.58%), RBC (30.53%), serum total protein (32.72%), albumin (28.44%) and globulin (38.01%) along with significant ($p<0.05$) elevation of serum MPO activity (83.30%), when compared with control. In addition, DIC increased gastric H_2O_2 and MDA levels by 33.93 and 48.59%, respectively, while the duodenal levels of the same parameters increased by 19.43 and 85.56%, respectively. Moreover, SOD, GPx and GST activities in the DIC group were significantly ($p<0.05$) reduced in the stomach (21.12, 24.35 and 51.28%, respectively) and duodenum (30.59, 16.35 and 37.90%, respectively), compared to control. Treatment with Gly and L-Arg resulted in significant amelioration of the DIC-induced alterations although L-Arg produced better amelioration of RBC (29.78%), total protein (10.12%), albumin (9.93%) and MPO (65.01%), compared to the DIC group. The protective effects of both amino acids against oxidative stress parameters and histological lesions were largely similar.

Conclusions: The data from this study suggest that Gly or L-Arg prevented DIC-induced gastro-duodenal toxicity and might, therefore be useful in improving the therapeutic index of DIC.

Keywords: antioxidants; arginine; gastrointestinal tract; glycine; NSAID; oxidative stress.

***Corresponding author:** Dr. **Akinleye Stephen Akinrinde**, Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria, Phone: +234(0) 7064368126, E-mail: as.akinrinde@gmail.com. https://orcid.org/0000-0001-6883-4595
Halimot Olawalarami Hameed, Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

Introduction

Non-steroidal anti-inflammatory drug (NSAID)-related gastrointestinal toxicity is a common clinical complication affecting large segments of populations, as the drugs are frequently prescribed for over-the-counter dispensing [1]. Diclofenac (DIC), like most traditional NSAIDs, produces analgesic and anti-inflammatory effects via inhibition of cyclooxygenases (COX 1 and 2), enzymes that catalyze the synthesis of prostanoids from arachidonic acid [2]. The non-selective inhibition of constitutively expressed cytoprotective prostaglandins involved in the maintenance of gastrointestinal mucosal integrity characterizes the acute and chronic toxicity of these drugs, giving rise to pathologic manifestations such as bleeding, peptic ulceration, hepatotoxicity and renal failure [3].

In addition to the inhibition of COX enzymes, DIC is known to produce toxicity via increased generation of reactive oxygen species (ROS), arising either from alteration of mitochondrial function or the metabolism of DIC via oxidative hydroxylation by cytochrome P450s (CYP 2C9 or 2C11) [4]. The resulting reactive metabolites, such as 4′-hydroxydiclofenac and 5-dihydroxydiclofenac, may undergo further oxidation to *p*-benzoquinone imines, which react with glutathione or microsomal proteins to produce oxidative stress [5]. Currently available therapies against NSAID-gastroenteropathy include concomitant use of proton pump inhibitors (PPIs), glucocorticoids and the development of selective COX-2 inhibitors [6], although

these approaches still pose a variety of side effects [7, 8]. Thus, there is continued search for novel therapeutic agents against NSAID-induced gastro-enteropathy. In this regard, the role of exogenous supplementation with biologically active dietary nutrients on the promotion of gut health is being increasingly recognized [9–11].

Glycine is the simplest non-essential amino acid synthesized endogenously from serine. It is an important substrate for the synthesis of several biomolecules such as porphyrins, glucose, neurotransmitters and purine nucleotides [12]. As a component of the tri-peptide glutathione, glycine (Gly) is utilized in the biochemical detoxification of endogenous toxins or xenobiotics via conjugation reactions [13]. Glycine was reported to exhibit anti-inflammatory and cyto-protective effects against intestinal injury induced by mesenteric ischemia and reperfusion [14]. Arginine, a nitric oxide (NO) donor, is often regarded as a semi-essential amino acid as although, synthesized in the body, its production is often insufficient to meet the body×s needs [15]. Biologically, arginine is important as a precursor of NO via oxidation reactions resulting in the release of NO and citrulline in a reaction catalyzed by Nitric oxide synthases (NOSs) [16]. Arginine-mediated synthesis of physiologic mediators such as NO, gastrin and polyamines is believed to underlie some of its beneficial effects in the gastrointestinal tract [17, 18].

The relative advantage of exploring the use of dietary supplements such as amino acids over other chemicals in ameliorating organ injury resides in the belief that they exhibit a generally higher safety profile [15]. The present study was, therefore, undertaken to investigate the effects of Gly and L-Arginine (L-Arg) supplementation on DIC-induced alterations in a rat model of NSAID-induced gastroenteropathy.

Materials and methods

Chemicals

L-Arge, Glycine, reduced glutathione (GSH), 1, 2-dichloro-4-nitrobenzene (CDNB), thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium hydroxide, xylenol orange, potassium hydroxide and hydrogen peroxide were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). Diclofenac sodium (Voltaren®) (2-[{2, 6-dichlorophenyl} amino] benzene acetic acid) was purchased from a reputable pharmacy in Ibadan, Nigeria. All other chemicals were of the highest purity commercially available.

Experimental animals

This study utilized 42 male Wistar rats (n=42), two to three months old, weighing 100–170 g which were obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The animals were kept in plastic cages in a well-ventilated animal house and were allowed to acclimatize for a week to the environmental conditions (12:12 h light-dark photoperiod and about 60% humidity). They were fed a standard pelleted diet and water *ad libitum*. All the experiments were performed in accordance to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” [19], published by the National Institute of Health. The experimental protocols used in the current study were approved and followed the institutional guidelines for animal welfare established by the Animal Care Use and Research Ethics Committee (ACUREC) of the University of Ibadan, Nigeria.

Preparation of compounds and experimental design

L-Arg, Gly and DIC were suspended in normal saline (0.9% NaCl) for administration to the rats in accordance with the body weights. The treatment groups consisted of six groups with seven rats each as follows:

- **Group A (Control):** Vehicle (normal saline; 0.9% NaCl) for eight days.
- **Group B (DIC only):** Vehicle (first five days) plus DIC (9 mg/kg, per os) twice daily for the final three days.
- **Group C (DIC + Gly1):** Gly (250 mg/kg) (first five days) plus co-treatment with DIC (9 mg/kg, per os) twice daily for the final three days.
- **Group D (DIC + Gly2):** Gly (500 mg/kg) (first five days) plus co-treatment with DIC (9 mg/kg, per os) twice daily for the final three days.
- **Group E (DIC + L-Arg1):** L-Arg (200 mg/kg) (first five days) plus co-treatment with DIC (9 mg/kg, per os) twice daily for the final three days.
- **Group F (DIC + L-Arg2):** L-Arg (400 mg/kg) (first five days) plus co-treatment with DIC (9 mg/kg, per os) twice daily for the final three days.

The experimental design was based on a model of NSAID gastroenteropathy reported by Singh et al. [20] with slight adjustments. Unlike the Singh et al.’s model, where DIC was administered for five days, the present study utilized a three day DIC administration because of the observation of death of rats from the fourth day during an initial pilot study. The dosages and duration of administration of Gly [21] and L-Arg [22] were selected based on previous studies.

Sample collection and preparation

About 24 h after the last administration, blood samples (about 3 mL) were collected from all the rats under Xylazine/Ketamine anesthesia from the retro-orbital venous plexus into heparinized and non-heparinized tubes for determination of hematological and serum biochemical parameters. Blood in non-heparinized tubes was allowed to clot for about an hour after which it was centrifuged at 1,790 ×*g* for 10 min at room temperature (23–25 °C). The clear supernatant was collected as serum and was used to estimate the serum protein profile (total protein and albumin). Rats were thereafter euthanized by cervical dislocation and the abdomen was opened up. The stomach was removed and opened along the greater curvature, while the duodenum was opened along the entire length to remove the contents.

The tissues were rinsed in ice-cold normal saline (0.9% NaCl) and blotted on dry filter paper. The tissues were divided into two parts: the larger part was reserved for biochemical studies, while a smaller portion was immediately transferred into 10% phosphate-buffered formalin for histopathological examination. Tissues for biochemical assays were homogenized in Tris-HCl buffer (50 mM, pH 7.4) containing 1.15% KCl. The homogenates were thereafter centrifuged in a cold centrifuge (4 °C) for 10 min at 10,000×*g* to separate the cytosolic fraction.

Estimation of hematological and serum biochemical parameters

The packed cell volume (PCV) was determined by the microhaematocrit centrifugation technique [23]. Hemoglobin (Hb) concentration was measured spectrophotometrically by the cyanmethaemoglobin method [23], while Red Cell count (RBC) were determined using the new improved Neubauer hemocytometer. Haematimetric indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentrations (MCHC) were calculated from the PCV, Hb and RBC values. Serum levels of total protein and albumin were determined using the Biuret method as described by Okutucu et al. [24]. Serum globulin was calculated as the difference between total protein and albumin values, while the albumin: globulin ratio (A:G) was also calculated. Serum NO level was measured as the content of nitrites in the tissues according to the method described by Olaleye et al. [25], while serum myeloperoxidase (MPO) activity was determined according to the method of Xia and Zweier [26], an index of systemic inflammation.

Markers of oxidative stress and antioxidant status

The cytosolic fraction obtained after centrifugation of tissue homogenates was used in the assay of biochemical parameters of oxidative stress (hydrogen peroxide, H_2O_2; malondialdehyde, MDA and reduced glutathione, GSH) and antioxidant enzymes (superoxide dismutase, SOD; glutathione peroxidase, GPx and glutathione S-transferase, GST). The total protein content of the tissues was determined using the Biuret method as described by Gornal et al. [27], using a standard curve prepared with Bovine serum albumin. Generation of H_2O_2 in gastric and duodenal tissues was measured spectrophotometrically according to the method of Wolff [28]. The tissue concentration of MDA was used as an index of lipid peroxidation, according to the method described by Varshney and Kale [29]. Tissue GSH concentration was measured using the method of Jollow et al. [30] using sulfosalicylic acid (4%, w/v) as protein precipitating agent and DTNB (5,5′-Dithiobis-(2-nitrobenzoic acid)) as the sulfhydryl group-reactive agent.

The activity of SOD was determined using the method of Misra and Fridovich [31]. The assay utilized the inhibition of the auto-oxidation of epinephrine in an acidic medium to adrenochrome by enzyme protein present in the samples. The absorbance was read at 480 nm and the values expressed as units per mg protein, where one unit describes SOD activity required to cause 50% inhibition of the auto-oxidation of epinephrine. The activity of GPx was measured using the method described by Rotruck et al. [32], which involved estimation of the concentration of GSH consumed in a reaction utilizing enzyme activity in the tissue samples. The activity of GST was estimated by the method of Habig et al. [33] using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate.

Histopathology

At termination of the experiments, small portions of the stomach and duodenum were immediately transferred to 10% phosphate-buffered formalin for histopathological examination. The tissues were later embedded in paraffin wax and sections (5–6 mm) were made. Staining was done with Hematoxylin and Eosin before light microscopic evaluation of the tissues [34].

Statistical analysis

Data were expressed as mean ± standard deviation and analyzed using GraphPad Prism software (Version 7.00). The differences among means were obtained with One way Analysis of Variance (ANOVA) followed by the Tukey's post hoc test for multiple comparisons across the groups. p values < 0.05 were considered statistically significant.

Results

Glycine or L-Arginine supplementation improved erythrocytic parameters in Diclofenac-treated rats

The effects of Gly and L-Arg on the erythrocytic indices of DIC-treated rats are presented in Table 1. Administration of DIC alone resulted in significant ($p<0.05$) decline in PCV (13.82%), Hb (46.58%) and RBC (30.53%) levels compared to the control group. In contrast, treatment with Gly at 250 and 500 mg/kg produced significant ($p<0.05$) improvement in Hb (14.53 and 24.30%, respectively) and RBC (18.53 and 23.75%, respectively) levels, compared to the DIC group. Similarly, when compared to the group treated with DIC alone, L-Arg produced increases in Hb (23.50 and 12.44%, respectively) and RBC (3.17 and 29.78%) values at the 200 and 400 mg/kg dosages, respectively. The improvements observed in these parameters were all statistically significant ($p<0.05$), except for RBC values in the group treated with L-Arg at 200 mg/kg. Generally, treatment with either Gly or L-Arg did not produce statistically significant ($p<0.05$) differences in PCV levels compared to those treated with DIC alone with the PCV in all the DIC-treated groups being generally lower than that of the control group. DIC treatment produced significant ($p<0.05$) increase in MCV (40.58%), while MCH (3.76%) and MCHC (18.98%) values were significantly ($p<0.05$) reduced, compared to the control group. Treatment with Gly2 (500 mg/kg) significantly ($p<0.05$) increased the DIC-induced reductions in MCV (29.81%) and MCHC (21.53%), while MCH

Table 1: Effects of Glycine and L-Arginine treatment on Diclofenac-induced changes in erythrocytic parameters.

Treatment groups	PCV, %	Hb, g/dL	RBC, ×10^6/μL	MCV, fl	MCH, pg	MCHC, g/dL
Control	38.00 ± 4.10	14.75 ± 1.41	6.37 ± 1.72	46.58 ± 6.64	33.01 ± 8.29	33.77 ± 2.54
DIC only	32.75 ± 3.20[a]	7.88 ± 0.20[a]	4.88 ± 0.50[a]	65.48 ± 2.98[a]	31.77 ± 3.17	27.36 ± 4.42[a]
DIC+ Gly (250 mg/kg)	30.00 ± 5.10[a]	9.22 ± 0.61[a,b]	5.99 ± 1.31[b]	49.42 ± 7.28[b]	35.98 ± 2.79	26.58 ± 3.22[a]
DIC+ Gly (500 mg/kg)	28.80 ± 4.49[a]	10.41 ± 1.19[a,b]	6.40 ± 1.10[b]	45.96 ± 15.20[b]	30.12 ± 7.38	33.25 ± 3.09[b]
DIC+ L-Arg (200 mg/kg)	32.25 ± 2.50[a]	10.30 ± 0.31[a,b]	5.04 ± 0.48	61.56 ± 9.75[a]	38.25 ± 4.46[b]	28.49 ± 5.22
DIC+ L-Arg (400 mg/kg)	34.40 ± 3.65[a]	9.00 ± 0.32[a,b]	6.95 ± 1.76[b]	60.05 ± 3.58[a]	34.61 ± 4.87	27.75 ± 2.63

PCV, Packed cell volume; Hb, Hemoglobin concentration; RBC, Red blood cell counts; MCV, Mean Corpuscular volume; MCH, Mean Corpuscular Hemoglobin, MCHC, Mean Corpuscular Hemoglobin Concentration. All data are expressed as the mean ± standard deviation (n=7). [a]Significant (p<0.05) compared to the control group. [b]Significant (p<0.05) compared to the DIC group.

(20.40%) value was significantly (p<0.05) increased in the L-Arg1 (200 mg/kg) group, relative to the DIC group.

L-Arginine but not glycine improved serum protein profiles of Diclofenac-treated rats

Similar to observations in erythrocytic indices, there was significant (p<0.05) decline in serum levels of total protein (32.72%), albumin (28.44%), globulin (30.01%) and the albumin-globulin ratio (12.35%) in the DIC-treated rats compared to the control group (Table 2). However, significant (p<0.05) increase in total serum protein and albumin levels was observed with L-Arg treatment at both 200 mg/kg (8.17 and 5.96%, respectively) and 400 mg/kg (10.12 and 9.93%, respectively), when compared to the DIC group. In contrast, treatment with Gly did not result in significant improvement of total protein, albumin and globulin levels compared to the DIC group, as values remained significantly lower than those of controls. Thus, it appears that L-Arg was more effective in restoring serum protein levels following DIC-induced losses of serum protein.

Glycine or L-Arginine treatment was effective in inhibiting oxidative stress and improving antioxidant enzymatic activities

As presented in Figure 1, the gastric and duodenal levels of H_2O_2 and MDA were significantly (p<0.05) elevated after treatment with DIC compared with the control group. Compared with the control group, gastric and duodenal levels of H_2O_2 in the DIC group increased by 33.93 and 19.43%, respectively, while MDA levels increased by 48.59 and 85.56% in the stomach and duodenum, respectively. Moreover, DIC produced significant (p<0.05) reduction in duodenal GSH (16.35%) compared with the control group, although gastric GSH levels remained unaltered. However, treatment with Gly2 (500 mg/kg) and L-Arg2 (400 mg/kg) significantly (p<0.05) decreased gastric H_2O_2 levels by 18.37

Table 2: Effects of Glycine and L-Arginine treatment on Diclofenac-induced changes in serum protein levels.

Treatment groups	Total protein, g/dL	Albumin, g/dL	Globulin, g/dL	A:G ratio, g/dL
Control	7.64 ± 0.54	4.22 ± 0.39	3.42 ± 0.31	0.81 ± 0.09
DIC only	5.14 ± 0.19[a]	3.02 ± 0.22[a]	2.12 ± 0.08[a]	0.71 ± 0.07[a]
DIC+ Gly (250 mg/kg)	5.38 ± 0.30[a]	3.04 ± 0.42[a]	2.34 ± 0.40[a]	0.79 ± 0.23
DIC+ Gly (500 mg/kg)	5.46 ± 0.48[a]	3.08 ± 0.37[a]	2.38 ± 0.33[a]	0.82 ± 0.11
DIC+ L-Arg (200 mg/kg)	5.56 ± 0.27[a,b]	3.20 ± 0.26[a]	2.36 ± 0.36[a]	0.75 ± 0.16
DIC+ L-Arg (400 mg/kg)	5.66 ± 0.48[a,b]	3.32 ± 0.26[a,b]	2.34 ± 0.34[a]	0.71 ± 0.10

All data are expressed as the mean ± standard deviation (n=7). [a]Significant (p<0.05) compared to the control group. [b]Significant (p<0.05) compared to the DIC group.

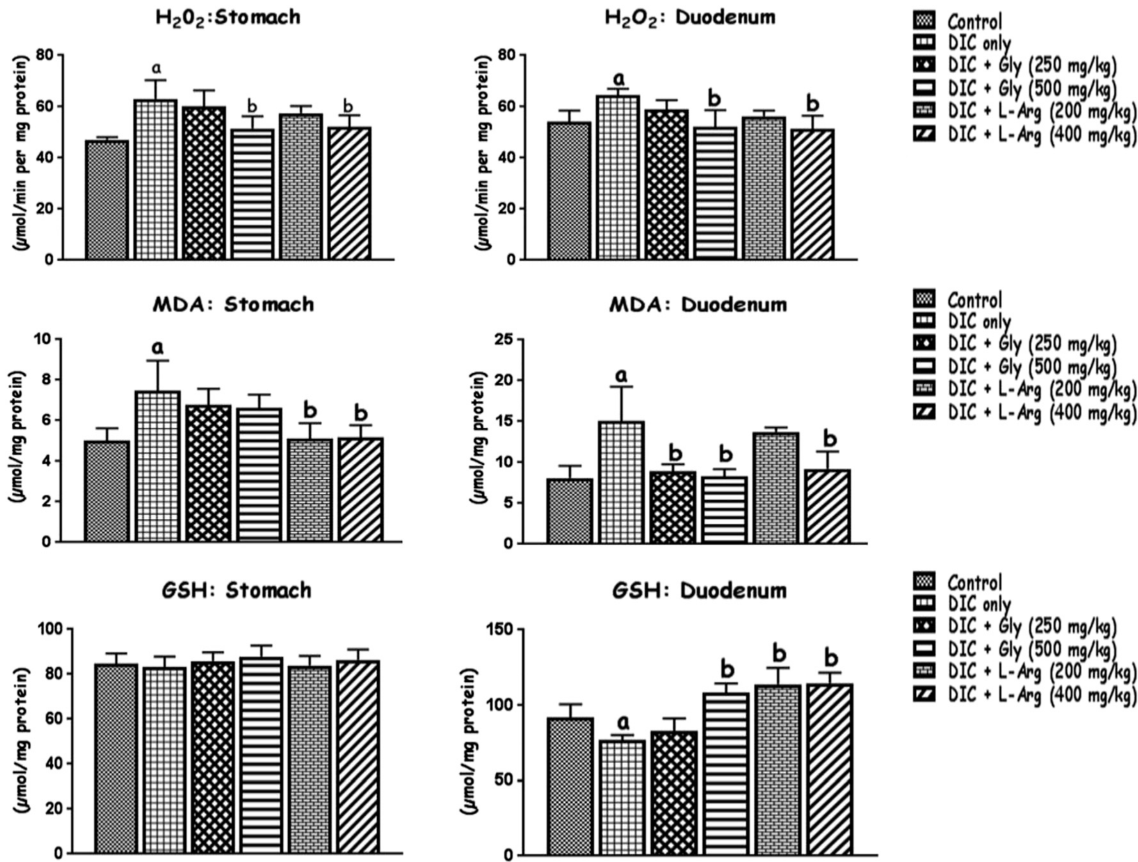

Figure 1: Glycine and L-Arginine supplementation attenuated oxidative stress markers, Hydrogen peroxide (H_2O_2), Malondialdehyde (MDA) and reduced glutathione (GSH) in gastric and duodenal tissues of Diclofenac-treated rats.
All data are expressed as the mean ± standard deviation (n=7).
[a]Significant (p<0.05) compared to the control group.
[b]Significant (p<0.05) compared to the DIC group.

and 17.55%, respectively, in comparison to the DIC group. Similarly, duodenal H_2O_2 levels were significantly ($p<0.05$) reduced by 19.07 and 20.33% with administration of Gly 2 (500 mg/kg) and L-Arg 2 (400 mg/kg), respectively, when compared to the DIC group.

Furthermore, gastric MDA levels were significantly ($p<0.05$) reduced when DIC was co-administered with L-Arg1 (31.24%) or L-Arg2 (30.63%), relative to the DIC group. Similarly, MDA levels in the duodenum reduced significantly ($p<0.05$) with administration of Gly1 (40.81%), Gly2 (45.06%) and L-Arg2 (38.58%) in comparison with the DIC group. In addition, co-administration of DIC with Gly2 (500 mg/kg), as well as L-Arg (200 and 400 mg/kg) significantly ($p<0.05$) improved the duodenal GSH levels by 41.31, 48.62 and 49.53%, respectively when compared with the DIC group. Summarily, both amino acids appeared to exhibit similar efficacy towards improving the DIC-induced alterations in oxidant status of gastric and duodenal tissues.

The activities of antioxidant enzymes SOD, GPx and GST measured in the gastric and duodenal tissues are presented in Figure 2. DIC treatment resulted in significant ($p<0.05$) decline in the activities of SOD, GPx and GST in both gastric and duodenal tissues, compared to the control group. With DIC administration, gastric SOD, GPx and GST activities decreased by 21.12, 24.35 and 51.28%, respectively, compared to the control. In similar fashion, duodenal SOD, GPx and GST activities reduced by 30.59, 16.35 and 37.90%, respectively in relation to the control group. However, co-treatment with L-Arg at 200 and 400 mg/kg significantly ($p<0.05$) improved gastric SOD activity by 27.01 and 48.56%, respectively when compared to the DIC group. Moreover, both Gly2 (500 mg/kg) and L-Arg2 (400 mg/kg) produced significant ($p<0.05$) increase in duodenal SOD activity by 38.63 and 37.55%, respectively.

Co-administration of DIC with Gly2 (500 mg/kg) (41.31%) and L-Arg (200 and 400 mg/kg) (48.62 and 49.53%, respectively) produced significant ($p<0.05$) enhancement of

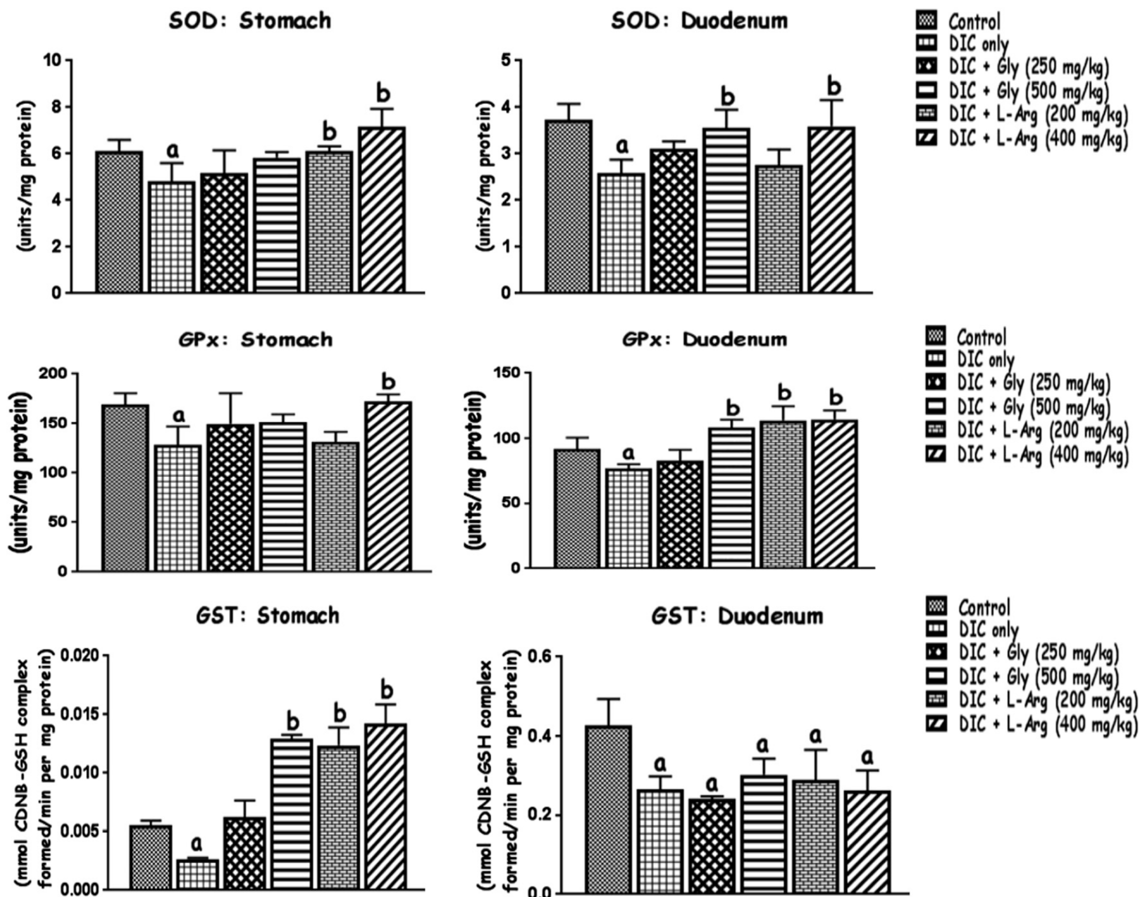

Figure 2: Glycine and L-Arginine supplementation improved the activities of antioxidant enzymes, superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Glutathione S-transferase (GST) in gastric and duodenal tissues of Diclofenac-treated rats.
All data are expressed as the mean ± standard deviation (n=7).
[a]Significant (p<0.05) compared to the control group.
[b]Significant (p<0.05) compared to the DIC group.

duodenal GPx activity compared to the rats treated with DIC alone. It was observed, however, that only the treatment with L-Arg2 (34.45%) was effective in improving gastric GPx activity, compared to the DIC group. Furthermore, there was significant (p<0.05) increase in gastric GST activity by Gly2 (381.01%), L-Arg1 (360.54%) and L-Arg2 (429.41%), compared to the DIC group. It appears from the results, therefore, that while both amino acids were effective in improving the tissue antioxidant status in stomach and duodenum, L-Arg could be effective even at lower doses compared to Gly.

Glycine and L-Arginine treatment was effective in attenuating serum inflammatory markers

Serum MPO activity and NO concentration were measured to reflect the systemic inflammatory status of the rats following the various treatments. As shown in Figure 3, DIC treatment produced significant (p<0.05) elevation in serum MPO activity by 83.30%, compared to the control. However, supplementation of rats with Gly2 (500 mg/kg) produced significant (p<0.05) reduction in MPO activity by 57.57%, compared to the DIC group. Administration of L-Arg at 200 and 400 mg/kg resulted in significant (p<0.05) inhibition of MPO activity by 64.66 and 65.01%, respectively, compared to the DIC group. This implies that L-Arg was more effective in normalizing the inflammatory alterations induced by DIC administration. Nevertheless, there were no treatment-related changes in the concentration of NO concentrations across all the groups.

Glycine or L-Arginine significantly improved gastric and duodenal morphology

Representative photomicrographs of gastric and duodenal mucosal sections in the various groups are depicted in

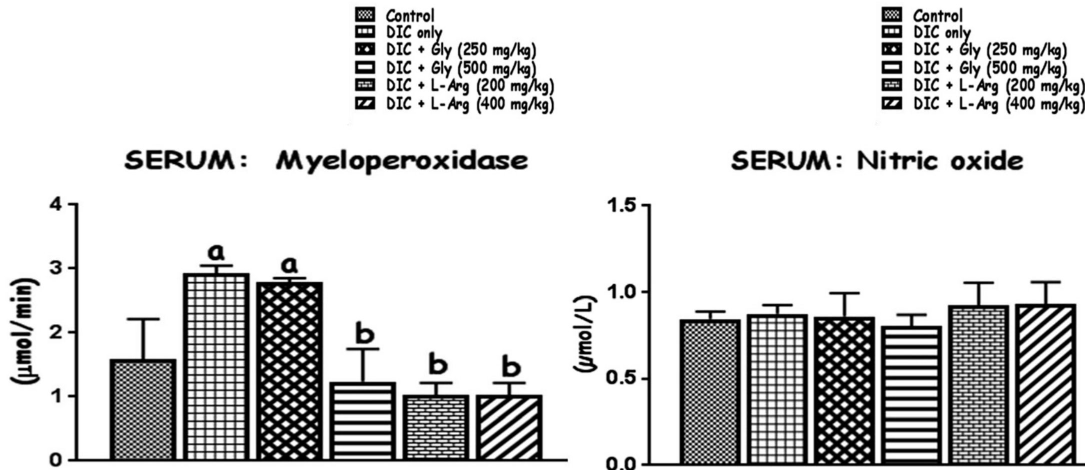

Figure 3: Effects of Gly and L-Arg on serum myeloperoxidase (MPO) activity and Nitric oxide (NO) concentration in Diclofenac-treated rats. All data are expressed as the mean ± standard deviation (n=7).
[a]Significant ($p<0.05$) compared to the control group.
[b]Significant ($p<0.05$) compared to the DIC group.

Figures 4 and 5. Sections from the control rats showed normal morphology with no visible lesions. DIC-induced lesions included severe erosions and focal necrosis of the gastric mucosa (Figure 4), while the duodenal sections showed extensive erosion and shortening of the villi (Figure 5). Administration of Gly at 250 mg/kg failed to significantly improve the gastric erosions. However, supplementation with Gly2 (500 mg/kg) and L-Arg (200 and 400 mg/kg) produced significant amelioration of mucosal pathology as no lesions were observed in the gastric mucosa of the rats given the respective treatments. Similarly, duodenal sections from rats treated with the different doses of Gly or L-Arg showed markedly improved morphology with normal villi architecture (Figure 5).

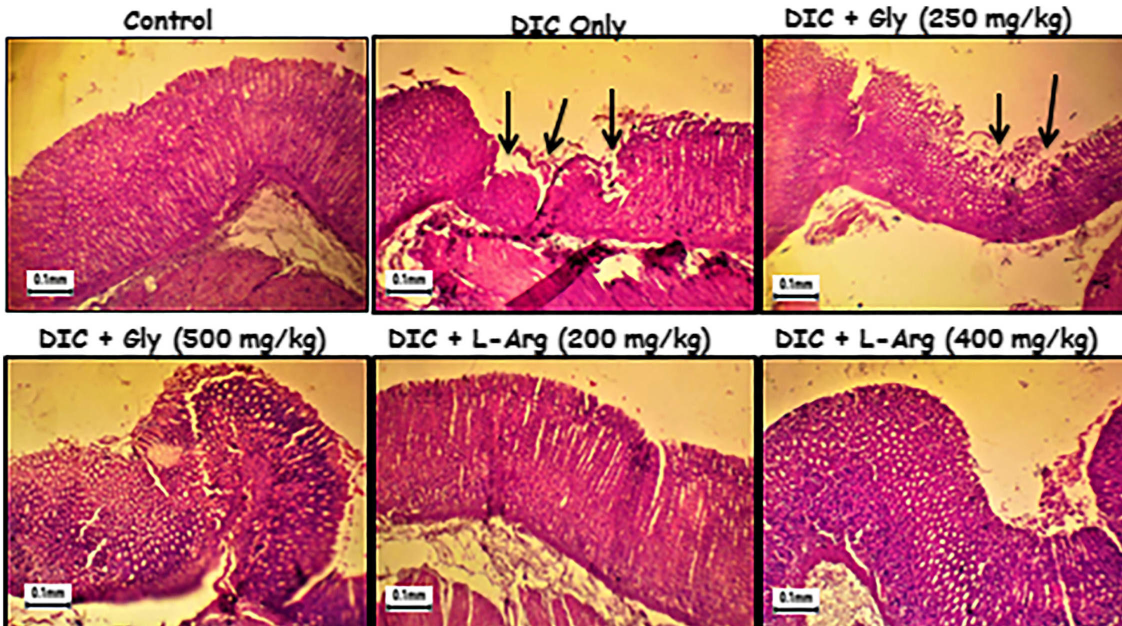

Figure 4: Representative photomicrographs of stomach sections in rats exposed to Diclofenac and treated with Glycine and L-Arginine. H&E; Mag. X 150. Arrows show focal necrosis and erosion of the mucosa. Scale bar 0.1 mm (100 µm).

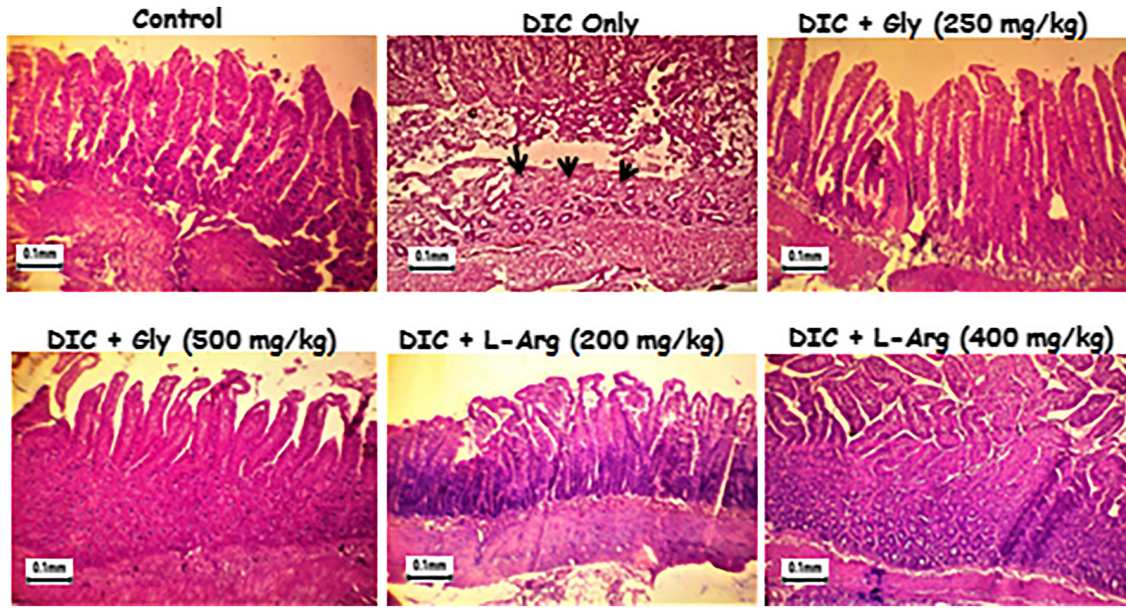

Figure 5: Representative photomicrographs of duodenal sections in rats exposed to Diclofenac and treated with Glycine and L-Arginine. H&E; Mag. X 150. The duodenal sections showed extensive erosion and shortening of the villi (black arrows) in the DIC group, which were ameliorated by Glycine and L-Arginine. Scale bar 0.1 mm (100 μm).

Discussion

Gastrointestinal damage caused by NSAIDs, such as DIC, remains a significant clinical problem [35]. The inhibition of cyclooxygenases and the induction of oxidative stress are the central mechanisms believed to be responsible for the gastrointestinal injury exerted by these drugs [3]. There is increasing evidence supporting a protective role of amino acids against drug-induced upper gastrointestinal tract pathology [36]. The present study was, therefore, designed to evaluate the potential protective properties of the amino acids Gly and L-Arg against DIC-induced injuries in the stomach and duodenum using a rat model.

In this study, rats treated with DIC manifested signs of gastrointestinal bleeding with dark and pasty diarrhea and evidence of haemorrhages in the gastric and intestinal mucosa (data not shown). In line with this, the analysis of erythrocytic indices showed significant decline in PCV, Hb and RBC levels following DIC administration when compared to the control group. These alterations are indicative of drug-induced toxicity and anemia which may be due to loss of erythrocytes and other blood components during gastrointestinal bleeding induced by DIC [37, 38]. Reduced Hb concentration following NSAID, and indeed DIC therapy, is generally associated with overt or occult blood loss (hemorrhage) [39]. Loss of Hb is a direct consequence of erythrocyte loss and it has been reported that a drop in PCH and Hb levels can be noticed as quickly as 10 min following and haemorrhagic episode [40].

In this study, the RBC picture showed increase in MCV and decreased MCHC which may suggest macrocytic and hypochromic type of anemia. The results are consistent with those previously reported by Singh et al. [20] which reported reductions in the same hematological parameters following DIC administration.

Further evidence of DIC-induced toxicity obtained from blood analysis include significant reductions in serum total protein, albumin and globulin levels, in all the groups exposed to DIC as compared to the control group. Reduced serum protein levels may be a result of reduction in protein synthesis caused by impairment of liver function [41]. Moreover, reduced serum protein levels may also result from gastrointestinal hemorrhage due to DIC toxicity. Similar results were also obtained by Singh et al. [20], who utilized similar dosage regimen of DIC as in the present study. Interestingly, treatment of rats with Gly or L-Arg resulted in significant improvement in Hb levels and RBC counts and suggests that the amino acids effectively inhibited DIC-induced blood and/or erythrocyte loss. Comparatively, both amino acids appeared to exert similar impact on the improvement of erythrocytic parameters during DIC administration, although Gly may appear to produce better preservation of erythrocytic morphologic indices. The requirement of the α-carbon of Gly for heme

synthesis in vertebrates has long been established. The synthesis of a single heme molecule normally requires one atom of iron and eight molecules of Gly with succinyl Co A generated from the citric acid cycle [42]. The observed increases in Hb and RBC values may, therefore, imply an active role for Gly in the synthesis of heme, and hence hemoglobin. The improvement of Hb values by L-Arg could be related to its involvement in synthesis of the globin polypeptides [43], prevention of DIC-induced hemolytic activities or protection of red cell membranes via enhancement of GSH synthesis [44].

In the present study, DIC caused significant increases in the oxidative parameters, H_2O_2 and MDA, while decreasing GSH concentration, as well as the activities of SOD, GPx and GST, all of which indicates a state of oxidative stress in the tissues. In addition, DIC administration induced significant increase in serum MPO activity, suggesting an increase in neutrophil activation and a systemic inflammatory state. The overproduction of ROS, such as H_2O_2 may contribute to the DIC-induced gastrointestinal damage by promoting both lipid and protein oxidation [45]. While DIC-induced oxidative stress has been widely reported [46], the stimulation of MPO activity by an anti-inflammatory drug appears to be an unexpected finding, as DIC may be expected to rather cause down-regulation of inflammation via inhibition of the release of prostaglandins. However, similar findings have been recorded by Halici et al. [47] who reported that DIC administration to rats resulted in exacerbation of MPO activity in carrageenan-induced inflammation in rats, although the reason for this finding is still unclear. Meanwhile, Zhang et al. [48] also indicated that MPO levels tend to increase concomitantly with gastric injury in indomethacin-treated rats.

Endogenous antioxidants, including enzymatic and non-enzymatic systems, play important roles in the protection of cells from ROS-mediated injuries [49]. During toxic exposures, however, the maintenance of these antioxidant systems may be dependent on exogenous supplementation with sources of antioxidants to achieve cellular homeostasis. In the present study, supplementation of rats with Gly or L-Arg resulted in significant restoration of the antioxidant pool, including GSH, SOD, GPx and GST. Although, the relative actions of these amino acids was slightly different between the stomach and duodenum, it can be reasonably inferred that their antioxidant-enhancing properties might be involved in suppressing oxidative parameters, including H_2O_2 and MDA levels in these tissues. Our findings are consistent with previous studies indicating the antioxidant-stimulating properties of these amino acids. For instance, Liang et al. [50] reported that L-Arg supplementation induced antioxidant response against oxidative stress by stimulating glutathione synthesis via activation of glutamate-cysteine ligase expression and the Nrf2 pathway. Similarly, antioxidant responses following Gly supplementation have been previously reported [51].

Histological analysis of the gastric and duodenal mucosa of the experimental rats indicated changes that corroborated the findings from biochemical analysis, and suggests that the induction of oxidative stress and/or depletion of antioxidant capacity in DIC-treated rats contributed significantly to the observed damage. As observed in the present study, DIC caused distortion of normal mucosal architecture, producing extensive erosion of gastric and duodenal mucosa with associated epithelial necrosis and haemorrhagic lesions. These changes are typical of NSAID-induced gastrointestinal toxicity as reported in previous studies [52]. The mucosal protective effects of Gly and L-Arg are likely related to their antioxidant-stimulating activities, with particular reference to their profound ability to inhibit lipid peroxidation.

Conclusions

Oral supplementation of rats with either Gly or L-Arg ameliorates DIC-induced gastro-duodenal toxicity by attenuation of oxidative stress and systemic inflammation, as well as, restoration of antioxidant capacity of the tissues. Therefore, Gly and L-Arg hold potential as adjuvant supplements for the management of NSAID-induced gastrointestinal toxicities.

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