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Research Article

Effect of Vitamins C & E on Aspirin Induced Gastric Mucosal Damage and Oxidative Stress

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Abstract: The effects of Vitamins C & E on Aspirin - induced gastric mucosal damage on gastric ulcer parameters and stomach oxidative stress markers were determined in acute and sub-acute studies in Wistar rats. Aspirin produced a significant ($p<0.05$) increase in gastric ulcer score in both studies. Vitamins C & E conferred protection in acute and sub-acute studies with preventive indices of 54 and 60% respectively. Histologically the gastric mucosa of animals in the sub-acute study showed a severe necrosis of the epithelial cells than observed in the acute study. Acute aspirin administration did not increase the stomach tissue Malondialdehyde (MDA) but sub-acute administration significantly ($p<0.05$) increased MDA, while vitamins C & E caused a significant ($p<0.05$) decrease below the level seen in normal controls. Aspirin in both studies significantly decreased Catalase activity. While acute aspirin exposure had no effect on Superoxide Dismutase (SOD) activity, sub-acute exposure raised it significantly ($p<0.05$). Administration of Vitamins C and E significantly increased SOD activity only in the acute study. Aspirin significantly decreased reduced Glutathione (GSH) in both studies and was reversed by Vitamin C and E. The level of GSH reductase (GSHRD) in the acute study was significantly decreased by aspirin but sub-acutely and prior treatment with vitamins C and E had no significant effect. Combine administration of vitamins C & E prior to intake of aspirin significantly prevented aspirin-induced gastric ulceration with decrease in some oxidative stress markers.

Keywords: Acetylsalicylic acid, antioxidants, free radicals, gastric ulcer

INTRODUCTION

Non-steroidal Anti-Inflammatory Drugs (NSAIDs) are the most commonly prescribed drugs because of their well-established efficacy in the treatment of pain, fever, inflammation and rheumatic disorders. Their use has increased due to the use of aspirin in the prophylaxis of ischemic heart disease and thrombotic disorders (Dalen, 2006; Nema *et al*., 2009). However, aspirin and other NSAIDs are associated with the occurrence of adverse Gastrointestinal (GI) side effects, ranging in severity from dyspeptic symptoms, gastrointestinal erosions and peptic ulcers to more serious complications such as bleeding or perforation (García and Barreales, 2007).

The established evidence that endogenous prostaglandins deficiency is associated with NSAIDs-induced gastroduodenal damage has provided important clues for the design of new anti-inflammatory drugs, which were expected to reduce toxicity. However, some of these agents (selective inhibitors of COX-2), while exhibiting a somewhat less toxicity, are also found to be associated with significant cardiovascular toxicity (Hippisley-Cox and Coupland, 2005). Research for the possible additional factors that might be involved have reported the association of oxidative stress in NSAIDs-induced gastric damage but the mechanism involved is not clear.

Studies have shown that vitamin C and E individually protect against ASA-induced gastric damage due to their antioxidant activities (Konturek *et al*., 2006; Fesharaki *et al*., 2006). A combination of vitamin E and C due to the synergistic interaction between the two vitamins might enhance their antioxidant benefits.

The aim of this study was to evaluate the effect of combined administration of vitamins C and E in aspirin-induced gastric mucosal damage, oxidative stress and antioxidant status in Wistar rats.

MATERIALS AND METHODS

Drugs and chemicals: Acetylsalicylic acid, L-Ascorbic acid, Cimetidine, alpha-tocopherol and all other chemicals and reagent were of analytical standard purchased from Sigma-Aldrich Germany.

Animals: Wistar strain rats (130-150 g) of both sexes were allowed to acclimatize for two weeks before

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experiment; they were maintained in dry cages and fed with a commercial poultry feed (Vital feed limited, Jos, Nigeria) and water *ad libitum*.

Experimental procedure: A total of 100 wistar rats were used in the study. The study was divided into 2 experiments: acute and sub-acute studies consisting of 50 rats each. Each of the study consisted of five groups (n = 10).

Normal control (n = 10): They received distilled water (1 mL/kg) orally.

Aspirin only (n = 10): They received oral Aspirin only.

Vitamin C and E only (n = 10): They received oral vitamins C & vitamin E only.

Aspirin+Vitamin C & E (n = 10): They received vitamins C & E orally one hour prior to administration of aspirin.

Aspirin+Cimetidine (n =10): They received oral cimetidine one hour prior to administration of aspirin.

Acute study groups were treated with a single oral Aspirin (300 mg/kg b.wt) (Angelo *et al*., 2010). In the appropriate groups oral vitamins C and E (100 and 10 mg/kg b.wt, respectively) or cimetidine (50 mg/kg b.wt) was given one hour before challenge with oral Aspirin (300 mg/kg b.wt). All animals were fasted for 36 h prior to onset of experiment; they were sacrificed 3hrs after administration of aspirin.

The sub-acute study groups were treated with daily oral aspirin (150 mg/kg b.wt) (Fesharaki *et al*., 2006) for 2 weeks. In the appropriate groups daily oral vitamins C and E (100 and 10 mg/kg b.wt, respectively) or cimetidine (50 mg/kg b.wt) were given one hour before challenge with oral Aspirin (150 mg/kg b.wt). All animals were fasted for 36 h starting from day 12^{th}, but were given respective treatment and water *ad libitum*; they were sacrificed 3hrs after administration of aspirin on day 14^{th}.

Preparation of organ extracts: To exactly 1.0 g of stomach tissue (from five rats in each group) 10 mL of 0.01 M phosphate buffer (PH 7.0) was added and homogenized. Portion of the homogenate were centrifuged at 3000×g for 10 min and supernatant collected.

Assessment of gastric mucosal damage: The stomach of each of the remaining rats was excised and opened along the greater curvature, washed in running tap water and ulcer scoring was carried out using a 2x hand lens by a person unaware of the experimental procedure. Evaluation of the degree of ulceration was expressed in terms of: ulcer score, ulcer index, percentage ulceration and preventive index.

Ulcer score was calculated by dividing the total number of ulcers in each group by number of rats in that group (Robert *et al*., 1968). Ulcer index (U.I) was calculated by multiplying ulcer score x 100 (Radwan *et al*., 2003); the ulcer incidence (%) was calculated by dividing the number of animals with ulcer by the total number of animals and multiplying by hundred (Ohara *et al*., 1992) and the preventive index was calculated according to the method of Hano *et al*. (1976).

Histological examination: The stomach of rats after ulcer scoring were collected from each group and placed in 10% formalin, processed for routine paraffin blocking and H & E staining.

Oxidative stress markers: Lipid peroxidation in stomach tissue was measured using the thiobarbituric acid test, by the modified method of Niehaus and Samuelson (1968). 0.15 mL of tissue homogenate was mixed with 2 mL of (TBA-TCA-HCL) solution and placed in water bath at 90°C for 60 min. After cooling the absorbance of the pink supernatant (TBA-malonaldehyde complex) was then measured at 532 nm. Malonaldehyde formed was then calculated using an extinction coefficient of 1.56×105 per M.cm.

Catalase activity in the supernatant was measured using the method of Sinha (1972). Based on the reduction of Dichromate in acetic acid to chromic acetate, when heated in the presence of hydrogen peroxide. Catalase activity was determined from the concentration of H_2O_2 in the supernatant. Superoxide Dismutase (SOD) activity in tissue homogenate was determined by a method described by Fridovich (1986), based on the ability of SOD to inhibit auto oxidation of adrenaline at pH 10.2. Reduced Glutathione (GSH) concentration measurements was done according to Ellman (1959) as described by Rajagopalan *et al*. (2004), based on the reaction of 5, 5'-dithiobis nitro benzoic acid *(*DNTB) and Reduced Glutathione (GSH). Glutathione Reductase activity was measured according to the procedure of Hsiao *et al*. (2001).

Statistical analysis: Results obtained are expressed as mean S.E.M. Statistical analysis was performed using ANOVA and posthoc-tukey test using SPSS/version17.0 statistical package. A level of $p<0.05$ was defined as being statistically significant.

RESULTS AND DISCUSSION

The effect of vitamins C and E on ulcer parameters in aspirin-induced gastric mucosal damage was investigated in acute and sub-acute studies and the results are shown in Table 1 and 2, respectively. Aspirin produced significant ($p<0.05$) increase in the gastric ulcer scores when assessed 3 h and 14 days after administration. Pretreatment with oral Vitamins C & E

Table 1: Effect of vitamins C and E on ulcer parameters in acute aspirin-induced gastric mucosal damage in Wister rat

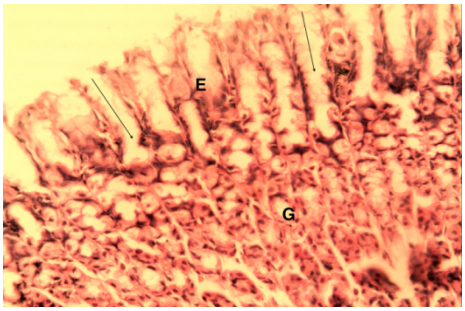
Ulcer parameters	Normal control	Aspirin only	Aspirin+Vits C and E	Vits C and E only	Aspirin+Cimetidine
Ulcer scores	0.20±0.00[a]	18.00±3.60[b]	8.33±0.88[c]	0.00±0.00[a]	2.33±1.45[ac]
Ulcer index	20	1800	833	0	233
Percentage ulceration	20%	100%	100%	0	40%
Preventive index	-	-	54%	100%	87%

Data are expressed as mean±SEM; values with different superscripts are statistically different along a row ($p<0.05$)

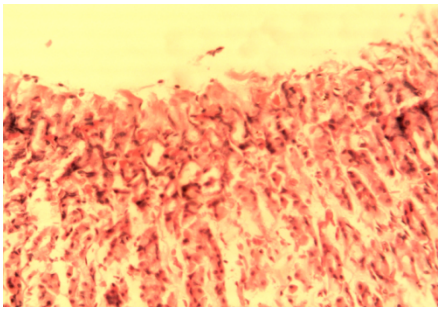
Table 2: Effect of vitamins C and E on ulcer parameters in sub-acute aspirin-induced gastric mucosal damage in Wister rat

Ulcer parameters	Normal control	Aspirin only	Aspirin+Vits C and E	Vits C and E only	Aspirin+Cimetidine
Ulcer score	0.60±0.4[a]	14.00±0.70[b]	5.60±0.60[b]	0.60±0.4[a]	3.20±0.80[b]
Ulcer index	60	1400	560	60	320
Percentage ulceration	40%	100%	100%	0	40%
Preventive index	-	-	60%	-	77%

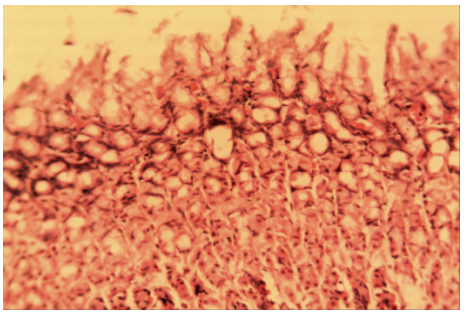
Data are expressed as mean±SEM; values with different superscripts are statistically different along a row ($p<0.05$)



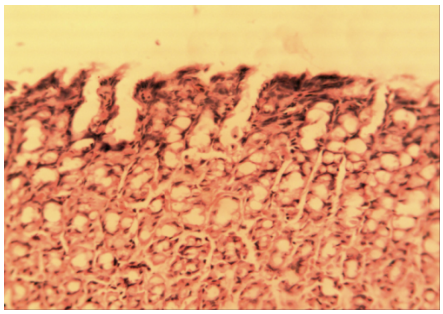
(a) Normal gastric mucosa



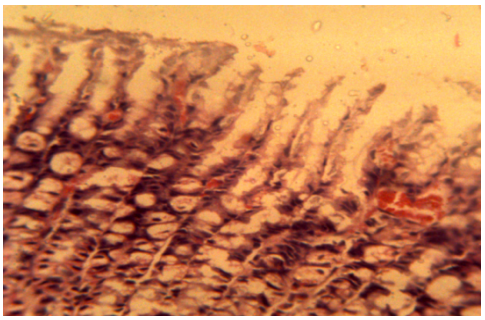
(b) Acute aspirin treated mucosa



(c) Aspirin+Vit C and E treated mucosa



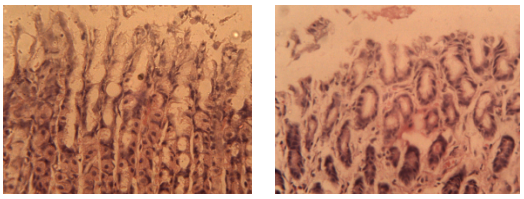
(d) Vit C and E treated mucosa



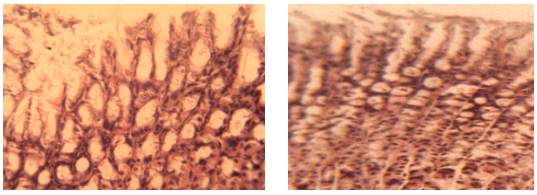
(e) Aspirin+ Cimetidine treated mucosa

Fig. 1: Photomicrogragph of gastric mucosa of rats treated with aspirin and/or vitamin C & E and cimetidine. H & E Stain x 250 (E-surface epithelial cells, G-gastric gland cells)

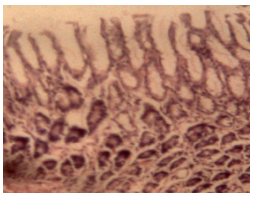
in combination at doses of 100 and 10 mg/kg, respectively, produced a significant decrease in the gastric ulcer scores with a preventive index of 54 and 60%, respectively. The antiulcer drug cimetidine (50



(a) Normal gastric mucosa (b) Sub-acute aspirin treated mucosa



(c) Aspirin+Vit C and E treated mucosa (d) Vit C and E treated mucosa



(e) Aspirin+Cimetidine treated mucosa

Fig. 2: Photomicrographs of gastric mucosa of rats treated with Aspirin and/or Vitamin C & E or Cimetidine. H & E Stain x 250 (E- surface epithelial cells, G-gastric gland cells)

mg/kg) in the acute and sub-acute studies inhibited ulcer formation with a preventive index of 87 and 77%, respectively. The observed effect of aspirin in the present study is consistent with previous reports showing that acute and/or chronic mucosal lesions of the stomach may result from oral ingestion of aspirin (Mabrouk *et al*., 2009; Angelo *et al*., 2010).

Gastrointestinal injury caused by Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) may result via topical (Musumba *et al*., 2009) and systemic action by blockade of cyclooxygenase-1 (Cox-1) and cyclooxygenase II (Cox-II) leading to prostaglandin depletion that may result in impairment of mucosal repair, facilitating mucosal injury (Burke *et al*., 2006). Acute aspirin-induced mucosal injury in the present study may as well be ascribed to the topical action of NSAIDs, while; the sub-acute effect might further involve the systemic action of the drug. Topical injury initiates the first mucosal erosion by disrupting the gastric epithelial cells as was observed in the photomicrographs (Fig. 1 and 2) which showed necrosis of the surface epithelia cells that were exposed to aspirin in the acute and sub-acute studies. Administration of vitamins C and E as well as cimetidine prior to aspirin caused less necrosis and inflammation of gastric glands in both studies.

The results of the present study indicates that a single oral dose of aspirin 300 mg/kg b.wt had no significant effect on stomach MDA level (Table 3), irrespective of gastric mucosal damage that occurred. But sub-acute (150 mg/kg b.wt) administration of aspirin for 2 weeks significantly increased MDA level as shown in Table 4. Prior treatment with vitamins C and E significantly decreased MDA level when compared with the aspirin only group inboth studies. As a lipid oxidation marker MDA is an index of oxidative stress, its accumulation indicates the presence of excess free radicals that cause oxidative stress, resulting in cell damage. Considering the association of oxidative stress with aspirin-induced gastric mucosal damage as was reported by Hsu *et al*. (2009) and Angelo *et al*. (2010), the increase in stomach MDA level in the present study was not unexpected. However, the occurrence of

Table 3: Effect of acute vitamins C & E on levels of oxidative stress markers in stomach tissue of acute aspirin-induced gastric mucosal damage in Wister rat

	Normal control	Aspirin only	Aspirin+Vits C & E	Vits C & E only	Aspirin+Cimetidine
MDA (µmol/g tissue)	5.00±0.37^{a}	5.16±0.35^{a}	2.08±0.16^{b}	1.88±0.08^{b}	2.52±0.02^{b}
Catalase (mol/min/g tissue)	0.28±0.04^{a}	0.19±0.01^{b}	1.18±0.07^{b}	0.41±0.04^{c}	0.33±0.01^{ac}
SOD (Units/g)*	21.10±0.00^{a}	21.05±0.01^{a}	25.27±1.21^{b}	73.70±0.61^{c}	71.70±0.81^{c}
GSH (µmol/g tissue)	0.11±0.01^{a}	0.07±0.00^{b}	0.09±0.00^{a}	0.09±0.01^{a}	0.13±0.02^{c}
GSHRd (nmol/min/g tissue)	0.02±0.01^{a}	0.01±0.00^{b}	0.01±0.00^{a}	0.50±0.01^{b}	0.01±0.00^{a}

Data are expressed as mean ±SEM; values with different superscripts are statistically different along a row ($p<0.05$); *1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 min

Table 4: Effect of sub-acute vitamins C & E on levels of oxidative stress markers in stomach tissue of sub-acute aspirin induced gastric mucosal damage in Wister rat

	Normal control	Aspirin only	Aspirin+Vits C & E	Vits C & E only	Aspirin+Cimetidine
MDA (µmol/g tissue)	12.13±0.41^{a}	25.29±0.65^{b}	11.00±0.89^{a}	7.23±0.66^{c}	6.11±0. 77^{c}
Catalase (mol/min/g tissue)	0.74±0.04^{a}	0.18±0.02^{b}	0.26±0.02^{c}	0.05±0.01	0.38±0.03^{c}
SOD (Units/g)*	1.55±0.04^{a}	1.67±0.04^{b}	1.85±0.03^{b}	1.50±0.03^{a}	1.65±0.04^{ab}
GSH (µmol/g tissue)	0.03±0.00^{a}	0.02±0.00^{b}	0.22±0.01^{c}	0.23±0.00^{c}	0.02±0.00^{b}
GSHRd (nmol/min/g tissue)	0.024±0.001^{a}	0.030±0.002^{a}	0.028±0.003^{a}	0.009±0.004^{b}	0.015±0.002^{c}

Data are expressed as mean±SEM; values with different superscripts are statistically different along a row ($p<0.05$). ; *1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 min

gastric damage with acute aspirin inspite of the unchanged MDA levels might be attributed to other mechanisms leading to aspirin-induced ulcers.

Antioxidant enzymes such as superoxide dismutase (SOD) and Catalase (CAT) are the first line of defense against Reactive Oxygen Species (ROS). Catalase is a hemeprotein that catalyzes the reduction of H_2O_2 to $2H_2O$ and O_2 and protects the tissue from highly reactive oxygen free radicals and hydroxyl radicals, while SOD is an important defense enzyme that catalyzes the dis mutation of superoxide anions into O_2 and H_2O_2 (Leopold and Loscalzo, 2009). In the present study the level of stomach CAT activity with acute and sub-acute exposure to aspirin was significantly decreased, prior treatment with vitamins C and E in combination significantly reversed the effect (Table 3 and 4). There was no significant effect on the level of SOD activity in the acute aspirin treatment but prior treatment with vitamins C and E increased it. Sub-acutely SOD level was significantly decreased and prior treatment with vitamins showed no significant effect. The decreased level of CAT and SOD activity observed with aspirin exposure in the present study might be attributed to O_2^- generating ability of aspirin (Hildeman *et al*., 2003). Also aspirin and its metabolite Salicylic Acid (SA) have been reported to have the ability to undergo hydroxylation, generating H_2O_2 (Kamble *et al*., 2013). It is thus likely that repeated administration of aspirin caused excessive generation of O_2^- and H_2O_2, resulting in decreased levels of CAT and SOD activities. The vitamins being antioxidants may have spared these enzymes; hence the increases observed in the vitamin-treated groups compared with the aspirin control group.

Reduced Glutathione (GSH) is one of the most abundant non-enzymatic antioxidant biomolecules present in the tissues. Its functions are removal of free oxygen species such as H_2O_2, superoxide anions and alkoxy radicals, maintenance of membrane protein thiols and to act as a substrate for GPx and Glutathione S- Transferase (GST) (Townsend *et al*., 2003). In the present study, the level of glutathione was significantly decreased in both acute and sub-acute aspirin- treated groups (Table 3 and 4) as was reported in other studies (Cuevas *et al*., 2011; Das and Roy, 2012). Moreover, decreased level of GSH is a characteristic feature of *OH-mediated oxidative damage of the gastric mucosa during ulceration (Bhattacharjee *et al*., 2000). Administration of vitamin C and E in combination significantly increased the GSH concentration in all cases, confirming their antioxidant action.

Acute aspirin caused a decrease in the activity of glutathione reductase (GSHRd) enzyme and prior treatment with vitamins C and E had no effect on the aspirin-induced drop in GSHRd (Table 3), while sub-acute administration as well as prior treatment with vitamin C and E showed no significant effect on stomach GSHRd (Table 4). In stressed animals the level of antioxidant enzymes generally drops (Townsend *et al*., 2003), this could explain the decrease in GSHRd because our data indicates that oral aspirin induced a decrease in CAT and GSH at acute and sub-acute doses. This result implies that combine administration of vitamins C and E is also protective against aspirin-induced gastric ulcers as were the individual vitamins, reported in previous studies (Cuevas *et al*., 2011).

CONCLUSION

Acute (high) dose of aspirin significantly induced gastric damage without lipid peroxidation but with decreases in endogenous antioxidant, like CAT, GSH and GSHRd. Whereas sub-acute low dose aspirin caused gastric damage with an increase in lipid peroxidation and decreases in CAT and GSH antioxidant. Vitamins C and E in combination prior to intake of aspirin significantly prevented aspirin-induced gastric ulceration and oxidative stress.

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