Original Research Article

De novo gastric acid secretion mediated by dietary acrylamide induced oxidative stress in stomach tissue of experimental rats

ABSTRACT

Background: Acrylamide input on gastric mucosa lesion is known but not fully elucidated. In this study the impact of dietary acrylamide on gastric acid secretion; an aggressive factor capable of causing erosion of the stomach tissue was evaluated to explain possible reason why acrylamide could induce gastric mucosa lesion. Thus, the study focuses on the impact of dietary acrylamide on gastric acid secretion and its association with mucosa lesion.

Materials and Methods: Fifteen (15) male Sprague-Dawley rats were grouped into three groups (n = 5). Group 1 (control) was fed with standard rat diet, Group 2 and 3 were fed with standard rat diet contaminated with acrylamide doses (7.5mg/kg and 15mg/kg respectively) reported to compromise gastric mucosa integrity. The experimental animals were allowed free access to their various feed and drinking water *ad libitum* for 4 weeks. Impact of the dietary acrylamide on gastric acid secretion, gastric acidity and stomach tissue oxidative stress biomarkers (lipid peroxidation (MDA), superoxide dismutase (SOD), Glutathione peroxidase (GPx), and Catalase, CAT) were determined.

Results: Average dietary consumption across the groups was 90.88% per week. Acrylamide contaminated diet significantly increased gastric acid secretion and gastric acidity in a dose dependent manner when compared to control, $P<0.01$. Dietary acrylamide also induced oxidative stress on stomach tissues by significantly increasing MDA as well as decreasing SOD, GPx, and CAT of the stomach in a dose dependent manner when compared to control, $P<0.01$.

Conclusion: Findings from the study suggests that oxidative stress induced on stomach tissue by dietary acrylamide could be as a result of the increase in gastric acid secretion and gastric acidity observed.

Keywords: Acrylamide, Dietary Acrylamide, Gastric acid secretion, Oxidative stress, Stomach tissue

1. Introduction

Gastric acid (gastric juice or stomach acid) is a gastro-aggressive factor of the stomach formed within the stomach lining [1, 2]; it plays a key role in protection of the gastric mucosa by preventing infectious agents from gaining access into the stomach since most bacteria cannot withstand its P^H and acidity [3, 4]. Gastric acid also play role in digestion of proteins by activating digestive enzymes that breaks down the long chains of its amino acids [5]. Gastric acid helps to neutralize and maintain the P^H of the stomach by interacting with bicarbonate in the mucous secreted by the mucous cells of the stomach [6, 7]. Gastric acid is referred to as gastro-aggressive factor because based on its acidic nature, it is suggested that prolonged increase in its secretion and acidity could lead to complication of the stomach as well as other organs in the body [8]. In this study, it was hypothesized that dietary acrylamide may have impact on gastric acid secretion and this may help elucidate certain aspect of reported gastric mucosa lesion induced by acrylamide.

Acrylamide, an industrial chemical used for manufacturing of personal care products, soil conditioners, wastewater treatment, paper and textile industries was reported to form in foods (snack foods, potato crisps, breads, cereal products, and coffee) prepared at high temperature via Maillard reaction[9-12]. Acrylamide has been reported to potentiate toxic and carcinogenic effects ranging from neurotoxicity, reproductive toxicity and others [12-15]. It has also been reported to cause gastric motility, gastric mucosa lesion and compromise gastric mucosa integrity [16-18]. Acrylamide input on gastric mucosa lesion is known but not fully elucidated. To further elucidate the reported input of acrylamide on stomach tissue; this study was designed to investigate the impact of dietary acrylamide on gastric acid secretion (a gastro-aggressive factor), its acidity as well as oxidative stress in stomach tissue; hence revealing potential route for food toxicity (acrylamide) on stomach.

2. MATERIALS AND METHODS

2.1. Experimental Design

Animals received humane care, kept in cages in a clean and comfortable environment under a dark/ light cycle. The animals (procured from the animal house of the Faculty of Basic Medical Sciences, Gregory University, Uturu) were acclimatized for a period of 14 days.

Fifteen (15) male Sprague-Dawley rats weighing about 150g – 210g were randomly grouped into three groups (n = 5). Group 1 (control) was fed with standard rat diet, Group 2 and 3 were fed with standard rat diet contaminated with acrylamide (Sigma Aldrich, China) doses (7.5mg/kg and 15mg/kg respectively) reported to compromise gastric mucosa integrity [19]. The experimental animals were allowed free access to their various feed and drinking water *ad libitum* for 4 weeks. Standard rat diets as well as drinking water were free from acrylamide.

2.2. Determination of Dietary intake

Various experimental feed includes three kinds of feed; standard diet free of acrylamide, standard diet contaminated with 7.5mg/kg of acrylamide and standard diet contaminated with 15mg/kg of acrylamide. The various feeds were served 50g daily. Dietary intake of the various feed was determined daily using electronic top loading balance (Mettler Toledo Series) to the nearest 0.01 g. It was expressed as;

W_D = W1 – W2, where W_D is dietary intake in grams; W1 is the weight of diet served (50g/24hrs) and W2 is the weight of diet remnant after 24hrs consumption.

Dietary intake was expressed in percentage (%) as; $W_D \div 100$

2.3.Determination of gastric acid secretion and gastric acidity

Gastric acid secretion was determined by pyloric ligation method [19, 20]. After dietary intake for 4weeks surgery was performed under ketamine anesthesia (40 mg/kg), the abdomen of each animal was opened through a midline epigastric incision, the stomach was exposed and the pyloric end was ligated with a fine thread tied round the pylorus, precaution was taken to avoid inclusion of adjacent blood vessels. The cut was closed with catgut and the animal returned to its cage for it to regain consciousness. 4 hours post-surgery, the rats was again anaesthetized, opened up and stomach tissues were removed after clamping the pylorus and the lower end of the esophagus. Gastric juice was collected by draining into a test tube and centrifuged at 1400g for 10min; supernatant volume and pH were recorded [21]. The gastric acidity of the gastric juice was determined by titrating to pH 7.0 with 0.01N NaOH, using phenolphthalein as indicator.

2.4. Determination of stomach tissue oxidative stress biomarkers

Stomach tissues (0.5g) harvested were homogenized on ice with ice-cold 0.1 M phosphate buffer (1: 4 w/v, pH 7.4); homogenates obtained was centrifuged at 2500 rpm for 10 min at 4^0C and the resulting supernatants was frozen at -4°C for further use. Aliquots of the supernatants were thereafter used for assays of oxidative stress biomarkers [19]; oxidative stress biomarkers (lipid peroxidation (MDA), superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase (CAT) were determined by method described by Nwosu et al., [22].

2.5. Statistical analysis

Statistical analysis was done using GraphPad Prism version 8; Results were expressed as mean ± standard error of mean (SEM). One way analysis of variance was used to determine the difference among various groups; multiple comparisons among groups was done using Boneferroni post hoc test and significance was considered at $p<0.05$.

3. RESULTS

3.1. Dietary intake

Average dietary consumption across the groups = 90.88% per week; G1 = 91.05% weekly, G2 = 90.95% weekly, G3 = 90.65% weekly.

Table 1: Weekly progression of dietary consumption across all groups

Groups	Week 1		Week 2		Week 3		Week 4		Average intake
	N	%	N	%	N	%	N	%	(%) per week
G1	45.1	90.2	43.8	87.6	44.3	88.6	48.9	97.8	91.05
G2	48.6	97.2	44.9	89.8	40.6	81.2	47.8	95.6	90.95
G3	41.9	83.8	47.2	94.4	45.5	91.0	46.7	93.4	90.65

N= average dietary intake grams per week, % = percentage of intake, G 1 = control standard diet, G2 = standard diet + 7.5mg/kg acrylamide, G3 = standard diet + 15mg/kg acrylamide.

3.2.Gastric acid secretions and gastric acidity mediated by dietary acrylamide

Consumption of dietary acrylamide (7.5mg/kg and 15mg/kg) significantly increased gastric acid secretion and gastric acidity (indicated by decreased pH) in a dose dependent manner when compared with control at P< 0.01. There was also significant difference on comparison between the impact of 7.5mg/kg and 15mg/kg acrylamide diet at P<0.001 (Figure 1).

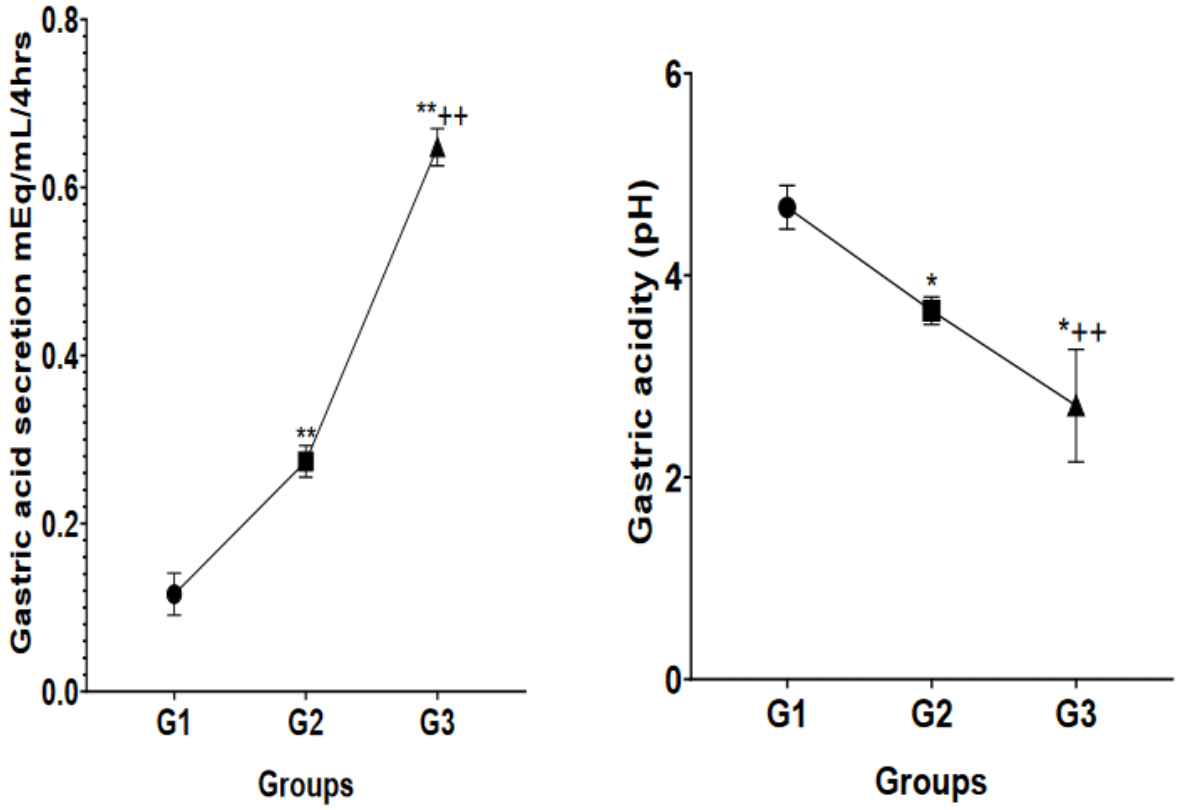

Figure 1: Values of gastric acid secretions and gastric acidity (pH) in all experimental groups. * indicate significant on comparing G2, G3 to G1 (** P<0.001, * P<0.01). + indicate significance difference between G2 and G3 (++ P<0.001, + P<0.01). G 1 = control (standard diet), G2 = standard diet + 7.5mg/kg acrylamide, G3 = standard diet + 15mg/kg acrylamide.

3.3.Oxidative stress in stomach tissue mediated by dietary acrylamide

Dietary acrylamide (7.5mg/kg and 15mg/kg) induced oxidative stress on stomach tissue by significantly increasing MDA as well as decreasing SOD, GPx and CAT in a dose dependent manner when compared to control diet at P<0.01. There was also significant difference on comparison between the impact of 7.5mg/kg and 15mg/kg acrylamide diet on stomach tissue oxidative stress biomarkers at P<0.01 (Figure 2).

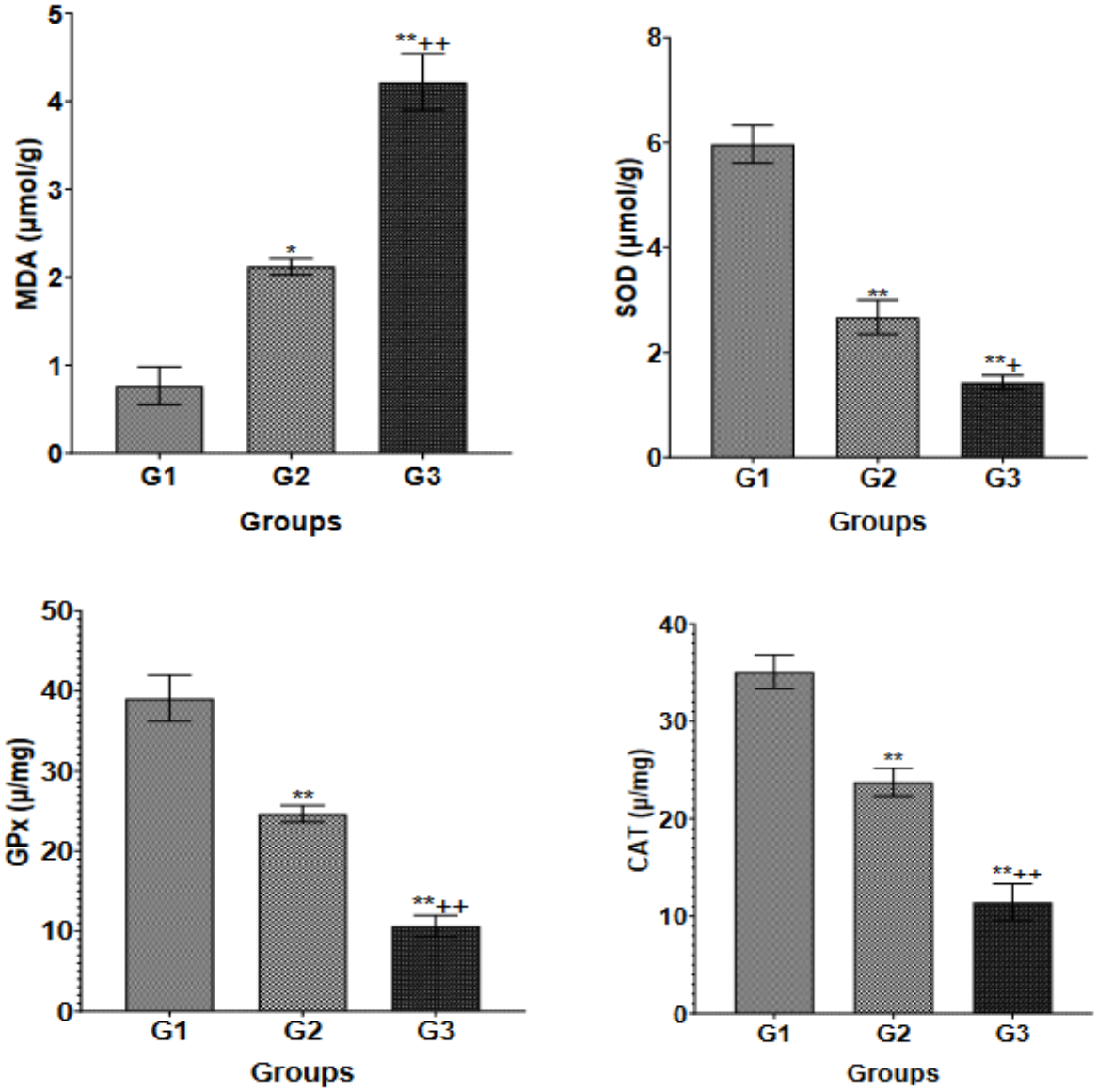

Figure 2: Values of oxidative stress biomarkers (MDA, SOD, GPx and CAT) in all experimental groups. * indicate significant on comparing G2, G3 to G1 (** P<0.001, * P<0.01). + indicate significance difference between G2 and G3 (++ P<0.001, + P<0.01). G 1 = control (standard diet), G2 = standard diet + 7.5mg/kg acrylamide, G3 = standard diet + 15mg/kg

acrylamide.

4. DISCUSSION

Dietary acrylamide (i.e. acrylamide formed in food when cooking, frying, toasting and baking high carbohydrate foods) [23] was mimicked in this study by contaminating standard rat diet with 7.5 mg/kg and 15mg/kg of acrylamide; these doses were used considering its reported deleterious input on gastric mucosa [19]. Average weekly dietary consumption of 90.88% was observed across the groups (Table 1) showing high level of consumption of the various diet including that of acrylamide diet; which suggests that the impact of dietary acrylamide observed on stomach tissue in this study was as a result of high percentage (Table 1) of acrylamide diet consumed. This corresponds to reports suggesting that increase intake of dietary acrylamide increases the risk of cells, tissues, organs and systems damage [24, 25].

Gastric acid is an aggressive factor capable of causing gastric mucosa erosion when there is imbalance between gastro-aggressive factor and gastro-protective factors like epithelial cells, mucus and bicarbonate concentration, prostaglandins, gastric mucosal blood flow, nitric oxide and antioxidants [19]. The result from this study suggests that dietary acrylamide increased gastric acid secretion (Figure 1) and decreased antioxidants of the stomach tissues (Figure 2) showing there is an increase in a gastro-aggressive factor and a decreased in gastro-protective factor, hence accounting for previous reported incidence of acrylamide-induced gastric mucosa lesion [16, 17].

The acidity of gastric juice accounts for its corrosive nature; the study showed that dietary acrylamide increased the acidity of the gastric acid secreted (Figure 1) which suggest that the corrosive nature of the gastric acid may have contributed to deleterious impact made on the stomach tissues as increased acidity of gastric juice has been reported to favor aggressive factors that predispose to gastric lesion [8].

Antioxidants of stomach tissues are among the gastro-protective factors that help to scavenge free radicals and protect stomach tissues from damage. Dietary acrylamide decreased antioxidants (SOD, CAT, GPx) and increasing lipid peroxidation (MDA) suggesting that dietary acrylamide induced oxidative stress to the stomach tissue, since increase in MDA and decrease in SOD, CAT and GPx has been associated to oxidative tissue damage [19, 22].

5. CONCLUSION

Dietary acrylamide induced de novo gastric acid secretion that could predispose to stomach tissue oxidative stress and damage, forming basis for understanding an aspect of gastric mucosa lesion induced by acrylamide.

Ethical Approval

This study was performed in accordance with the Ethics and Regulation Guiding the Use of

Research Animals as approved by Ethics Review Committee, Department of Human Physiology, Gregory University, Uturu, Nigeria.

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