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Research Article *Erratum***

Oral administration of acrylamide compromises gastric mucosal integrity in Wistar rats

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ABSTRACT

Background: Acrylamide, a potential toxicant and carcinogen, maybe formed in carbohydrate-rich food cooked at very high temperature. Its effect on gastric mucosa defense is not fully elucidated. Hence, the effect of acrylamide ingestion on gastric mucosal integrity was investigated. **Methods:** Fifty-four (54) Wistar rats (150-200g) were randomly divided into 3 groups; Group I (control) received 0.2mL distilled H_20, Groups II and III received 7.5mg/kg body weight and 15mg/kg body weight acrylamide respectively. Both acrylamide and distilled water were administered orally for 28days. Thereafter, gastric secretion was obtained and analysed for gastric acidity. Gastric antioxidants status (superoxide dismutase (SOD), reduced glutathione, catalase), lipid peroxidation, mucus content, nitric oxide, bicarbonate, prostaglandins-E and gastric mucus content were determined. Blood samples were also collected and evaluated for haematological indices. Histological changes, parietal and mucus cell counts were evaluated on gastric tissues. **Results:** Gastric secretion and acidity increased ($P < 0.05$) in the 15mg/kg acrylamide treated group. Glutathione, SOD, catalase, mucus content, bicarbonate, prostaglandins-E_2, mucous cell counts were reduced ($P < 0.05$) while parietal cell count, lipid peroxidation and nitric oxide increased ($P < 0.05$) in both acrylamide treated groups compared to control. White blood cell count in group II was increased compared to control ($P < 0.05$). Acrylamide treated groups displayed gastric epithelial cells with poor architecture, lamina propria, submucosa inflammatory cell infiltration and vascular congestion. **Conclusion:** Acrylamide exposure causes degeneration of gastric mucosal integrity in a dose-dependent manner via reductions in gastric protective factors, which thus predisposes the gastric mucosa to erosions and lesions.

INTRODUCTION

The gastrointestinal barrier has been described as a barrier between the body and a luminal environment that not only contains nutrients, but also is laden with potentially hostile microorganisms and toxins (Allaire *et al.*, 2018). When food is ingested, the gastrointestinal barrier acts as a first line of defence against invasion of foreign pathogens that might have been ingested (Hammer *et al.*, 2015) and disruption of this barrier has been reported to result in severe debilitating disease conditions (Allaire *et al.*, 2018). The gastric mucosa maintains its integrity by a balance between gastro-aggressive (acid and pepsin secretion) and gastro-protective factors (epithelial cells, mucus and bicarbonate concentration, prostaglandins, gastric mucosal blood flow, nitric oxide and antioxidants) (Goel *et al.*, 1985, Abdel-Salam *et al.*, 2001; Goel and Sairam, 2002). These factors constitute a complex system of interacting mediators that contribute to strengthening the gastric mucosa and offer resistance against gastric injury or insults.

Acrylamide is an industrial chemical used in the manufacture of personal care and grooming products, soil conditioners, wastewater treatment, as well as in paper and textile industries (Friedman, 2003; Exon, 2006). High levels of acrylamide have also been detected in tobacco smoke (Pruser and Flynn, 2011). Acrylamide is also a by-product of the cooking process having been reported to be a preparation by-product in

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heat-processed foods high in carbohydrates e.g. snack foods, potato crisps, breads, cereal products, and coffee (Mottram *et al.,* 2002). Acrylamide in diet is formed through the Maillard reaction where reducing sugars (glucose or fructose) react with the amino acid, asparagine. This reaction is responsible for browning food during baking, frying, and roasting of food (Mottram *et al.,* 2002). Therefore, it is likely that the general populace may be exposed to acrylamide through their diets.

Since its discovery in everyday foods (Pellucchi *et al.,* 2011; Virk-Baker *et al.,* 2014), several epidemiological studies have reported its potentially toxic and carcinogenic effects in different organs in the body (Mucci *et al.,* 2003; Hogervorst *et al.,* 2007; Hogervors *et al.,* 2008; Larsson *et al.,* 2009; Virk-Baker *et al.,* 2014). Acrylamide has also been reported to be a potent neurotoxin affecting both central and peripheral nervous systems (Lehning *et al.,* 2002; LoPachin *et al.,* 2002); however, its effect on the gastric intestinal tract has not been fully elucidated. While El-Mehi and El-Sherif, (2015) have reported acrylamide consumption causes mucosal erosions and depletion of the protective surface mucus, the underlying mechanism through which it disrupts the gastric mucosa defense is yet to be fully elucidated.

This study was therefore designed to evaluate the effect of acrylamide consumption on factors that maintain the integrity of the gastric mucosa.

MATERIALS AND METHODS

Animals and grouping

Fifty-four (54) male Wistar rats (150-170g) were housed in standard well-aerated laboratory cages and maintained at room temperature with alternating 12-hour day and night cycles. They were fed on standard rat chow and allowed free access to drinking water *ad libitum.* The animals were randomly divided into 3 groups of 18 rats each.

Treatment protocol

Group 1 - control received distilled water 0.2mLs, groups II and III received 7.5mg/kg body weight and 15mg/kg body weight of acrylamide (Sigma Aldrich, China) (Zenick *et al.,* 1986) respectively. All treatments were given orally for 28days. The Applied and Environmental Physiology Unit, Department of Physiology, University of Ibadan approved this experiment. Animals received humane care, and procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA).

Determination of gastric juice acidity and pH

Post-treatment, animals (n = 5) were subjected to surgery under light ether anaesthesia according to Brodie and Knapp (1966). Briefly, under light ketamine anaesthesia (40 mg/kg) the abdomen of each animal was opened through a midline epigastric incision, and the stomach was exposed. The pyloric end was identified and a fine thread was tied round the pylorus, care was taken to avoid inclusion of adjacent blood vessels. The wound was then closed with catgut and the animal returned to its cage where it subsequently regained consciousness. After 4 hours the animal was again anaesthetized, opened up and stomach was removed after clamping the pylorus and the lower end of the oesophagus. 4-hour gastric juice was collected and drained into a graduated test tube and centrifuged at 1400g for 10min (Raji *et al.,* 2011). The supernatant volume and pH were recorded (Saranya and Geetha, 2011) and the total acid content of the gastric juice collected was determined by titrating to pH 7.0 with 0.01N NaOH, using phenolphthalein as indicator.

Determination of ulcer score, gastric oxidative stress, bicarbonate and prostaglandins-E2 levels

Gastric ulcer score was done using a hand lens at X2 magnification as described by Elegbe and Bamgbose (1976) and thereafter the ulcer index and percentage (%) ulcer inhibition was calculated. Stomach tissues (0.5g) from 5 animals in each group were homogenized on ice with ice-cold 0.1 M phosphate buffer (1: 4 w/v, pH 7.4), the homogenates obtained was centrifuged at 2500 rpm for 10 min at 4^0C and the resulting supernatants was frozen at -4°C until use (Saheed *et al.,* 2015). Aliquots of the supernatants were thereafter analysed for catalase (Sinha, 1972), superoxide dismutase (SOD) (Misra and Fridovich, 1972), glutathione (Sedlak and Lindsay, 1968), lipid peroxidation (as malondyaldehyde (MDA) and nitric oxide (Griess reaction as described by Green 1982) levels respectively. The supernatants were also assayed for bicarbonate ion and prostaglandins-E2 level using enzyme-linked Immunosorbent Assay Kits (Bioassay Technology Laboratory, China).

Determination of haematological indices and mucus content in control and acrylamide treated animals

Blood samples were obtained by cardiac puncture after light ketamine anaesthesia (40 mg/kg) from 5 animals in each group into heparinised specimen bottles and analysed for packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), platelet count, total white blood cell (WBC) count and differential WBC count). Gastric mucous content was estimated in these same animals using the Alcian blue technique as described by Corne *et al.* (1974).

Parietal, mucous cell counts and Histological evaluation of the gastric mucosa

The stomach samples from animals in each group (n=3) were excised and stored in 10% formalin. Mucous cell count was estimated using the Periodic Acid Schiff (PAS) reaction technique while gastric histopathology and parietal cell count were estimated using Hematoxylin and Eosin-staining techniques as described by Adewoye and Salami (2013).

Statistical analysis

Results are expressed as mean ± SEM and were analysed using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test. Comparisons between control and experimental groups were carried out and the statistical differences were taken to be significant at $p < 0.05$.

RESULTS

Effect acrylamide on the gastric juice acidity and pH

The pH of gastric effluents in group III (acrylamide 15mg/kg treated)) was significantly reduced ($p<0.05$) compared to group I (3.56 ± 0.28 vs. 4.88 ± 0.29) while group II (acrylamide 7.5mg/kg treated) was not significantly different from control (4.44 ± 0.38) vs. 4.88 ± 0.29). Gastric Acid secretion (mEq/mL/4hours) in groups II (0.28 ± 0.04) and III (0.69 ± 0.12) were significantly increased ($p<0.05$) compared to control (0.07 ± 0.01) (Table 1).

Table 1. Effect of acrylamide on the gastric juice acidity and pH.

Groups	**Acidity (pH)**	**Gastric acid secretion (mEq/mL/4hours)**
Group I (Control)	4.88 ± 0.29	0.07 ± 0.01
Group II (Acylamide 7.5mg/kg treated)	4.44 ± 0.38	0.28 ± 0.04*
Group III (Acylamide 15mg/kg treated)	3.56 ± 0.28#	0.69 ± 0.12#

* Indicates significant differences between group II and control, # indicates significant differences between group III and control.

Gastric oxidative stress and bicarbonate level in control and acrylamide treated animals

Gastric antioxidants (superoxide dismutase (SOD), reduced glutathione and catalase were significantly reduced ($p<0.05$) in groups II (acrylamide 7.5mg/kg treated) and III (acrylamide 15mg/kg treated) compared to control. Gastric MDA (μmol/g) in groups III (0.234 ± 0.035) and II (0.059 ± 0.006) were significantly increased ($p<0.05$) compared to control (0.0177±0.002). Gastric bicarbonate (mmol/l) was significantly reduced ($p<0.05$) in groups III (4.05 ± 0.18 vs 7.66 ± 0.55) and II (4.85 ± 0.23 vs 7.66 ± 0.55) compared to control (Table 2).

Table 2. Effect of acrylamide on antioxidant status enzymes activities and lipid peroxidation parameter

Groups	SOD (μmol/g protein)	CAT (μmol/g protein)	GSH (μg/g)	HCO_3^- (mmol/L)	MDA (μmol/g)
Group I (Control)	6.18±0.89	28.54±1.89*	10.87±0.76	7.66±0.55	0.0177±0.02
Group II (Acrylamide 7.5mg/kg treated)	2.08±0.53*	19.14±1.86*	6.41±0.83*	4.85±0.23*	0.059±0.006*
Group III (Acrylamide 15mg/kg treated)	1.08±0.24#	15.18±1.67#	5.00±0.18#	4.05±0.18#	0.233±0.035#

* Indicates significant differences between group II and control, # indicates significant differences between group III and control.

Gastric ulcer score, index and inhibition in control and acrylamide treated animals

Gastric ulcer score was significantly increased ($p<0.05$) in group III (15mg/kg acrylamide treated) compared to control (group I) while values in group II (7.5mg/kg acrylamide treated) were not different from controls (Table 3). Ulcer index and percentage inhibition in group III was 0.49 and -88.46%, in group II it was 0.30 and -15.39% while in control it was 0.26 and 0% respectively (Table 3).

Table 3. Effect of acrylamide on ulcer score (units), ulcer index and ulcer inhibition

Groups	Ulcer score (units)	Ulcer index	% Ulcer inhibition
Control	5.1 ± 0.70	0.26	–
7.5mg/kg of acrylamide	6.0 ± 2.41	0.30	- 15.39
15mg/kg of acrylamide	9.8 ± 1.91#	0.49#	- 88.46

# Indicates significant differences between group III and I.

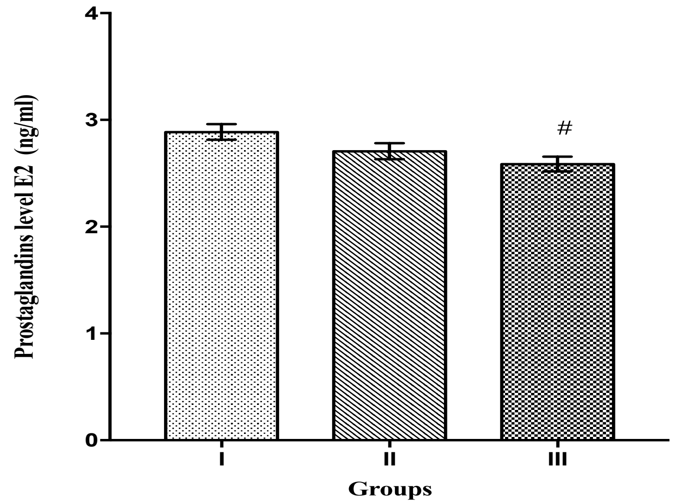

Fig. 2. Effect of acrylamide on gastric prostaglandin E2 level. #Indicates significant differences between group III and control. I = Control, II = Acrylamide (7.5mg/kg) treated, group III = Acrylamide (15mg/kg) treated group

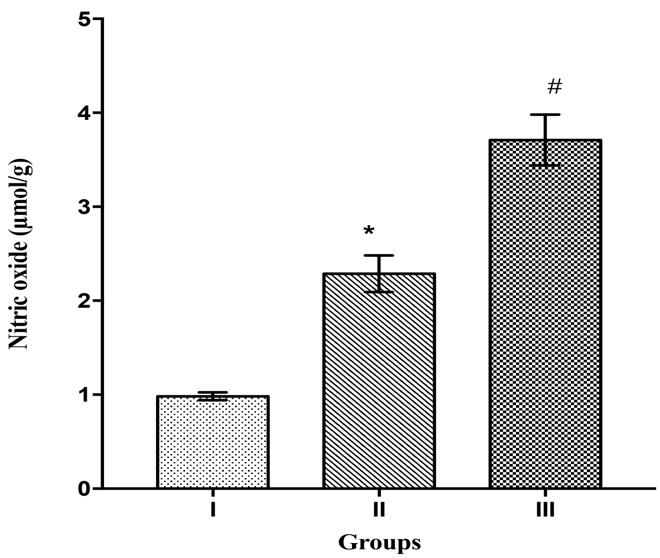

Fig. 1. Effect of acrylamide on gastric nitric oxide concentration. *Indicates significant differences between group II and control, # indicates significant differences between group III and control. I = Control, II = Acrylamide (7.5mg/kg) treated, group III = Acrylamide (15mg/kg) treated group

Gastric nitric oxide and prostaglandins E_2 levels in control and acrylamide treated animals

Gastric nitric oxide (µmol/g) was significantly increased in groups III (3.71 ± 0.27) and II (2.29 ± 0.20) compared to group I (0.98 ± 0.04) (Fig 1). Prostaglandins-E_2 (ng/mL) values in group III (2.59 ± 0.07) were significantly reduced while that in group II (2.71 ± 0.08) was not significantly different to group I (2.89 ± 0.07) (Fig. 2).

Gastric mucus concentration, parietal and mucous cell counts in control and acrylamide treated animals

Gastric mucus concentration (µg/g) was significantly reduced in both experimental groups compared to control (Fig. 3). Parietal cell count (cells /field) was significantly decreased ($p<0.05$) in group II (acrylamide 7.5mg/kg treated) and increased in group III (acrylamide 15mg/kg treated) compared to group I. Mucous cell count (cells /field) in groups II (486.0 ± 102.2) and III (361.7 ± 30.6) were significantly decreased compared to group I (814.7 ± 19.5) (Table 4).

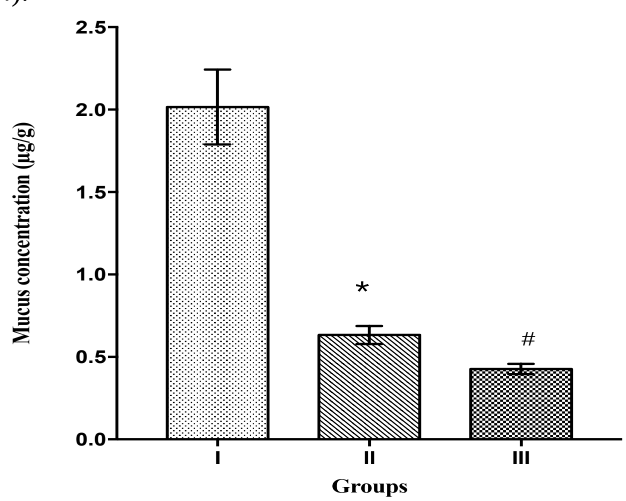

Fig. 3. Effect of acrylamide on gastric mucus concentration. *Indicates significant differences between group II and control, #indicates significant differences between group III and control. I = Control, II = Acrylamide (7.5mg/kg) treated, group III = Acrylamide (15mg/kg) treated group

Table 4. Effect of acrylamide on parietal and mucus cell counts

Groups	Parietal cell count cells/field)	Mucus cell count (cell/field)
Group I	419.3±7.84	814.7±19.5
Group II	385.7±5.24*	486.0±102.2*
Group III	492.3±8.29#	361.7±30.6#

* Indicates significant differences between group II and I, # indicates significant differences between group III and I.

Haematological indices in control and acrylamide treated animals

Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell counts (RBC) and platelet counts in all treatment groups were not significantly different from group I (Table 5). However, total white blood cell count (WBC) in group II (acrylamide 7.5mg/kg treated) (42.0 ± 3.51 x10^5) was significantly increased ($p<0.05$) compared to group I (32.6 ± 4.24 x10^5). Group III (acrylamide 15mg/kg treated) WBC counts were not significantly different from group I (Table 5). Monocytes and eosinophils in the experimental groups were not significantly different from control (group I) values. Lymphocyte values were significantly increased in group II (acrylamide 7.5mg/kg treated) (72.2±1.28) but decreased in group III (acrylamide 15mg/kg treated) (62.4±3.08) compared to control (67.6±1.03). Neutrophil count was significantly decreased in group II (low dose - 7.5mg/kg of acrylamide) (20.0±4.34) but increased in group III (acrylamide 15mg/kg treated) (35.4±3.20) compared to control (29.6±0.81) (Table 5).

Table 5: Effect of acrylamide on haematological indices

Haematological indices	Group I	Group II	Group III
PCV (%)	38.6±0.75	40.6±1.03	34.6±0.68
Hb (g/dL)	12.8±0.23	13.46±0.41	11.28±0.30
RBC count (10^{12}/L)	6.31±0.07	6.69±0.20	5.52±0.19
WBC count (10^9/L)	3.26±0.42	4.20±0.35*	3.36±5.85
Platelets (mm^3/L	15.84±1.96	9.86±0.78*	13.5±1.09
Neutrophil	29.6±0.81	20.0±4.34*	35.4±3.20
Lymphocyte (%)	67.6±1.03	72.2±1.28	62.4±3.31
Monocytes	1.8±0.37	1.8±0.37	1.4±0.25
Eosinophil	1.2±0.58	1.8±0.58	1.2±0.58

*Indicates significant differences between group II and I.

Histopathology of the gastric mucosa

The gastric mucosa of the control group (group I) had normal architecture, well preserved mucosa epithelial cells layer (white arrow) and the mucosa layer showed no infiltration of the gastric glands and lamina propria (slender arrow). The submucosal (blue arrow) and circular muscle (red arrow) layers were normal and were not infiltrated by inflammatory cells. Group II animals (Acrylamide 7.5mg/kg treated) had gastric mucosa with poor architecture, poorly preserved mucosa epithelial cell layer (white arrow) and mild infiltration of the lamina propria. The submucosal layer in this group had inflammatory cell infiltration, however the circular muscle layer appears normal. Group III (Acrylamide 15mg/kg treated) showed mucosa layer with eroded epithelial cells (white arrow), infiltrated lamina propria. The submucosal layers in this group appear moderately infiltrated by inflammatory cells (blue arrow) while the circular muscle layer appeared normal. Mild vascular congestion was also observed (Fig. 4 A-C).

DISCUSSION

Acrylamide has been described as a toxicant and an irritant (Zamani *et al.*, 2017). The discovery that it may be produced when cooking, frying, toasting and baking high carbohydrate foods has increased investigations into its potential biologic effects. These investigations have reported the neurotoxicity, reproductive toxicity and immune toxicity of acrylamide consumption (Zamani *et al.*, 2017. In this study the effects of acrylamide on gastric mucosal integrity was evaluated at two doses, 7.5mg/kg and 15mg/kg, which have been reported to be equivalent to 1/20 and 1/10 of LD_{50} for acrylamide (LD_{50} 150 mg/ kg) respectively (Zenick *et al.*, 1986). The significantly increased acidity and secretion of gastric juice especially in the high dose (15mg//kg acrylamide) compared to control (Table 1) suggests a predisposition of the treated animals to gastric ulceration as excess acidity of gastric juice has been reported to favour aggressive factors that predispose to gastric ulceration (Wormsley, 1974). Furthermore, parietal cells, which are responsible for acid secretion (Pavelka and Roth, 2010; Ige *et al.*, 2016), had increased counts in the high dose group compared to control (Table 4) suggesting a likely increase in gastric acidity and secretion in this group. This may thus be responsible for the significantly increased ulcer score and index seen in the acrylamide-exposed groups compared to control (Table 3).

Gastric antioxidants, an essential component of the gastrointestinal defence system that scavenge free radicals, have been reported to play an integral role in

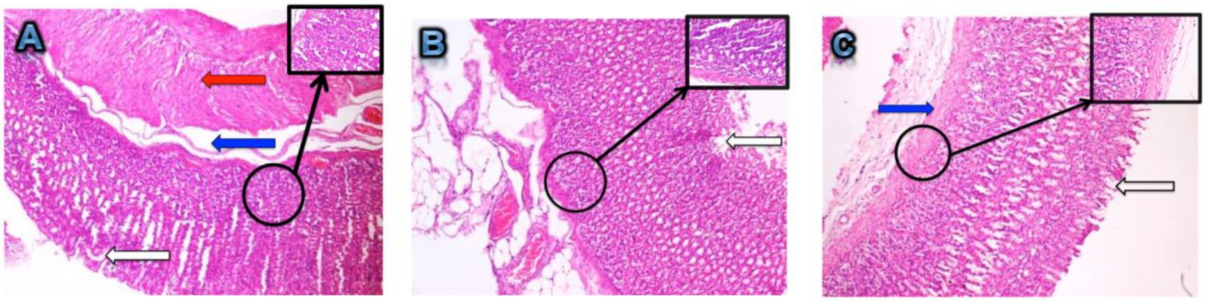

Fig. 4. (A-C) Photomicrograph of stomach samples in control and experimental groups at low magnification (x100) and high magnification (x400) Group 1 (Control) displayed normal architecture of gastric mucosa, with well-preserved mucosa epithelial cells layer (white arrow), the mucosa layer showed no infiltration of the gastric glands and lamina propria. The submucosal layers appeared normal and were not infiltrated by inflammatory cells (blue arrow), the circular muscle layer (red arrow) appears normal. Group 2 (Acrylamide 7.5mg/kg treated) exhibited poor architecture, the mucosa epithelial cells layer was poorly preserved (white arrow), and the mucosa layer displayed mild infiltration of the lamina propria and the gastric gland. The submucosal layers appear mildly infiltrated by inflammatory cells; the circular muscle layer appears normal. Group 3 (Acrylamide 15mg/kg treated) exhibited mucosa layer with eroded epithelial cells (white arrow), the mucosa layer shows mild infiltration of the lamina propria. The submucosal layers appeared moderately infiltrated by inflammatory cells (blue arrow), the circular muscle layer appeared normal. There is also was mild vascular congestion (Fig. 4, A-C).

the formation of gastric lesions (Hassan *et al.*, 1998). This study also shows depletion of gastric antioxidants and significant increase in gastric lipid peroxidationcompared to control (Table 2) suggesting a decline in the antioxidant capacity and increased oxidative stress in the gastric mucosa of the acrylamide exposed animals. Mucus, secreted by mucus cells, and bicarbonate ions secreted by gastric and duodenal epithelial cells constitute an integral component of the gastrointestinal barrier against erosion and invasion (Engle *et al.,* 1995). The mucus produced reduces the shear stresses on the epithelium and contributes to barrier function through various mechanisms, which include binding to bacteria thus preventing epithelial colonization and retarding diffusion of agents that can damage the epithelial surface e.g. acid secretion. Bicarbonate ion, on the other hand, serves to maintain a neutral pH along the epithelial plasma membrane, despite the highly acidic conditions existing in the gastric lumen (Engle *et al.*, 1995). This study shows a dose dependent and significant decrease in gastric bicarbonate concentration (Table 2), mucous content (Fig. 3) and mucous cell count (Table 4) compared to control which suggests an impairment in the ability of the gastric mucosa of the acrylamide treated animals to sustain its barrier function and prevent trans-epithelial migration of bacteria and antigens. It is thus likely that increased exposure to acrylamide enhances gastro-aggressive and suppresses gastro-protective factors that may predispose the stomach to gastric ulceration and lesions.

Inflammation within the gastrointestinal tract has been reported to result in the activation of inducible nitric oxide synthase (iNOS) leading to an increase in nitric oxide (NO) production that results in increased production of reactive oxygen radicals and oxidative stress (Muscara and Wallance, 1999; Lanas, 2008). An increase in NO was seen in the acrylamide-exposed groups compared to control (Fig. 1) and suggests a likely inflammatory mediated pathway for acrylamide-induced disruption of the gastric mucosa. Furthermore, prostaglandins whose gastro-cytoprotective effects are exerted by their ability to stimulate mucosal mucus and bicarbonate secretion, increase mucosal blood flow and partially limit back diffusion of acid into the epithelium (Wallance, 2008) was reduced in the high dose acrylamide group compared to control (Fig. 2) thus suggesting an impairment of prostaglandin enabled gastro-protection and increased susceptibility of the gastric barrier to damage.

Haematological and serum biochemical indices are important tools in evaluating the health status of an individual (Ige *et al.*, 2015). This study shows no significant difference in red cell indices (red blood cell count, packed cell volume and haemoglobin levels) across the groups (Table 5) which is consistent with Rawi *et al.*, (2012) who reported no change in haemoglobin, erythrocyte count and haematocrit levels in immature male rats and a decrease of these same indices in immature female following acrylamide (15mg/kg) treatment. This thus suggests the question of a likelihood of a gender effect regarding acrylamide toxicity and will form a subject for subsequent research in our laboratory. However, elevations in total white blood cell counts accompanied by reductions in neutrophil count were observed in the acrylamide treated, especially the low dose group, compared to control (Table 5). This suggests stimulation of the immune system arising from acrylamide exposure. Interestingly the high dose acrylamide group showed elevations in neutrophil counts and reduction in lymphocyte count compared to control, which suggests nutritional impairment and immune suppression in this group (Gonda *et al.*, 2017). Neutrophils are of particular importance to gastrointestinal integrity as diverse insults to the gastric mucosa, including infectious processes, ischemia and damaging chemicals have been reported to promote infiltration of the gastric mucosa by neutrophils (Gayle *et al.*, 2000). This study shows neutrophil infiltration of the gastric mucosa in groups 2(Fig 4B) and 3 (Fig 4C), which again suggest gastric tissue damage in the acrylamide-exposed groups. Furthermore, histological analysis in the different groups are consistent with the result of biochemical assays carried out and the report of El-Mehi and El-Sherif, (2015) who stated that acrylamide effects on the gastric mucosa include mucosal erosions, depletion of the protective surface mucus and inflammatory infiltration of the mucosal layer.

In conclusion, it may be inferred from this study that increased dietary acrylamide exposure, compromises the integrity of the gastric mucosal barrier by increasing the activity of gastro aggressive factors (decreased gastric acid pH and mucous cell count, increased gastric acid secretion, gastric lipid peroxidation, nitric oxide production, parietal cell and neutrophil counts respectively) and suppressing gastro protective factors (decreased gastric mucus, prostaglandins, antioxidants, bicarbonate ion. Hence, excessive browning while frying or toasting should be avoided as this causes acrylamide formation and accumulation in food, which may result to gastric mucosal damage or exacerbate already formed gastric ulcers.

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