Evaluating Effect of Prolonged Alcohol Consumption On Serum Gastrin and Secretin and Histo-archiecture of the Stomach and Duodenum In Rats

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Abstract

Alcoholic beverages are amongst the liquid most consumed globally and prolonged alcohol consumption can result in injuries to the gastrointestinal tract mucosa of an individual.It can also exhibit its effect on digestion and absorption. This research investigated the effects of long term varying doses of alcohol on two digestive hormones and the histological integrity of the stomach and duodenum in adult male *Wistar* rats. Forty adult male *Wistar*rats weighing 150-230g were used. The animals were randomly selected into eight groups comprising five rats each. Group 1 served as the control, while Groups 2, 3 and 4 received 0.5ml of 5%, 20% and 40% alcohol (0.05g/kg, 0.2g/kg and 0.4g/kg) respectively; Groups 5,6 and 7 were given 5% alcohol+Omega-3, 20% alcohol+Omega-3 and 40% alcohol+Omega-3 respectively while Group 8 was given Omega-3 alone. Omega-3 was administered at a dose of0.01ml of 0.2g/kg body weight. Treatment lasted for 12 weeks, following which serum gastrin and secretin levels, as well as the histology of the stomach and duodenum were analysed. Findings showed that serum levels of gastrin and secretin hormones increased with increasing alcohol concentration. Histology of the stomach and duodenum showed necrosis and atrophy of epithelial and submucous cells and this was more evident with higher alcohol concentration. Omega-3 was used as an ameliorating agent in this study to mitigate the effect of alcohol and result showed that its effect was dependent on alcohol concentration. This study concludes therefore that consumption of alcohol especially in high doses has the capacity to elevate serum levels of gastrin and secretin probably due to its ability to cause disruption of the architecture of the mucosa and submucosa of the stomach and duodenum as seen in this study. These deleterious actions of alcohol are often not ameliorated by use of anti-oxidants such as Omega 3 used in this study.

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Introduction

Alcohol ranks amongst the most abused drugs globally, but is not often thought of as a drug because of its use in religious and social settings in most parts of the world[1]. Based on their alcohol content, alcoholic beverages are categorized into three groups; wines, beers and spirits (gin or whisky). Fermentation forms the basis of all alcoholic beverages formation.

Alcohol is usually consumed orally and being the first point of contact, the gastrointestinal tract often bears the brunt of first pass phenomenon along with the liver. The effect of alcohol consumption on the intestinal tract often affects the health of an individual through its effect on digestion and absorption.[2] Alcohol has been known at high concentration to distort the mucosa of the oesophagus, stomach and small intestine, along with its deleterious effect on the pancreas and liver.[2,3]

In their study, Singer et al.[4] noted that intravenous, intragastric or alcohol ingestion at low concentrations of up to 5% stimulated gastric acid secretion, while higher doses either exerted no effect, or showed inhibitory actions. Same study also showed gastric emptying to be enhanced by low dose alcohol, while gastric emptying and motility were reduced by high dose. In their own study, Oluwoleet al.[5] found that an alcoholic beverage was able to enhance gastric mucus secretion. Alcohol consumption has frequently been identified as the cause gastritis in alcoholics and in individuals who regularly consume alcohol.

Some commonly observed symptoms amongst heavy alcohol users are diarrhea, and mal-absorption, occasioned by alterations in food digestion and absorption. Hence this study was carried out to investigate the effects of long term consumption of alcohol at varying concentrations 5%, 20% and 40% (0.05g/kg, 0.2g/kg and 0.4g/kg) respectively, on two digestive hormones, serum gastrin and secretin, while also examining the histological architecture of the stomach and small intestines.

Materials and Methods

Chemicals:

Analytical grade alcohol, Omega 3 fish oil supplement from BR Pharmaceuticals, Enzyme- linked immunosorbent assay kit from Hangzhou Eastbiopharm Co. Ltd (Xihu district, Hiangzhou

Zhejiang, China), 10% Formal Saline, Haematoxylin-Eosin Stain and Phenolphtalein indicator.

Reconstitution of Alcohol:

The various concentrations of alcohol were reconstituted as follows: 5% alcohol concentration was derived from 5ml absolute alcohol mixed with 95ml of distilled water, and the rest were derived similarly.

Experimental Animals:

Forty (40) male *Wistar* rats weighing 150–230g were used for this study. The rats were procured from the animal section of the Faculty of Basic Medical Sciences of Delta State University, Abraka. They were housed in metabolic cages, fed with commercial chow and allowed free access to drinking water. The animals were handled accordance to the guidelines for animal handling by Ward and Elsea.[6] Also adopted for this research was NIH Guide for Laboratory Animals Care and Utilization. The experimental protocol on handling laboratory animals was endorsed by the institutions ethical committee.

Experimental Protocol:

The rats were randomly selected into eight (8) groups of five (5) rats each (n = 5). The alcohol concentrations (5%, 20% and 40%), represents the level commonly associated with the frequently consumed alcoholic beverages namely beer, alcoholic wine, spirits (brandy, whisky, vodka or gin). The alcohol was purchased from an authorized pharmaceutical company (BR Pharmaceutical). The rats received single daily alcohol oral dose of 0.5ml of 0.05g/kg, 0.2g/kg and 0.4g/kg respectively, using an oro-gastric cannula. They also received 0.01ml of 0.2g/kg body weight of Omega-3 fatty acid. The treatment groups are as follows: Group 1, rats received only clean drinking water and normal feed, Group 2 rats received 0.05g/kg bw alcohol,Group 3 rats received 0.2g/kg bw alcohol, Group 4 rats received 0.4g/kg bw alcohol,Group 5 rats received 0.05g/kg bw alcohol plus omega-3 only,Group 6 rats received 0.2g/kg bw alcohol plus omega-3,Group 7 rats received 0.4g/kg bw alcohol plus omega-3 and Group 8 rats received only omega-3.The rats were allowed free access to food with water *ad libitum.* The treatment lasted for twelve weeks.

Sample Collection

At the end of twelve weeks, the final weights of all the animals were taken. They were fasted overnight and sacrificed via cervical dislocation. Laparotomy was done to reveal their internal organs. Blood was collected by cardiac-puncture into clean plain bottles and centrifuged at 4000rpm for 10min to obtain the serum. The stomachs and intestines were carefully harvested and rinsed with normal saline.

Assay of Serum Gastrin and Secretin:

Serum gastrin and secretin were measured with the aid of ELISA kits.

Histopathological Examination:

Samples of tissues from the stomach and duodenum of the rats were harvested. These were fixed in 10% formalin, dehydrated, cleared in xylene and embedded in paraffin. Afterwards, they were sectioned with the aid of a microtome to 5µm thick sections, stained with Haematoxylin-Eosin stain, and viewed under a light microscope. Finally, photomicrographs were taken.

Statistical Analysis

The results were expressed as Mean ± Standard Error of the Mean (SEM). The means of the treated and control groups were then compared using ANOVA with Fisher's Post-Hoc. P-values of less than 0.05 were considered statistically significant.

Results

Gastrin and Secretin

Result from this study showed an increase in serum gastrin with increasing alcohol concentration (Fig1). Co-administration of omega-3 with alcohol in all groups showed increased gastrin secretion compared to control and omega-3 alone. 0.05g/kg bw

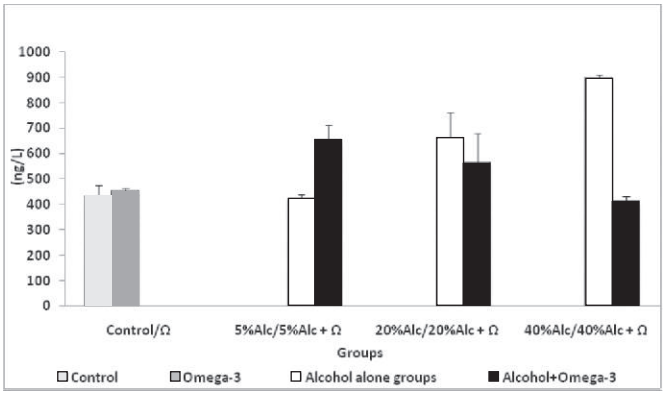

Fig.1: Effect of alcohol on the level of serumgastrin in experimental rats expressed as Mean±S.E.M. Keys: *Ω:* Omega-3, ALC: Alcohol,

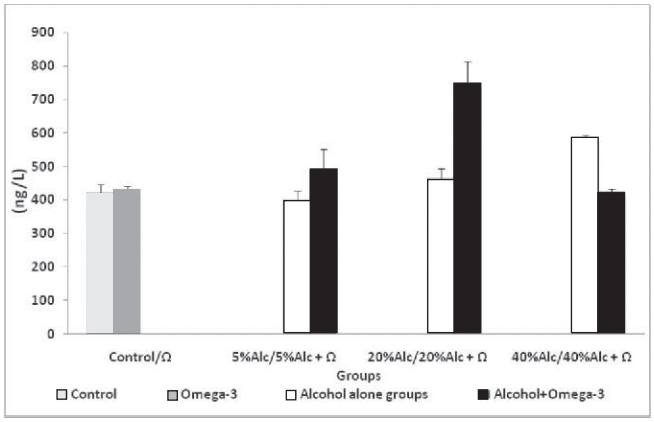

Fig. 2: Effects of alcohol on the level of serum secretin in experimental rats expressed as Mean±S.E.M. Keys: *Ω:* Omega-3, ALC: Alcohol

Effect of Alcohol Administration on Histomorphologic integrity of the stomach sections from Experimental Rats (figs 3a – 3g)

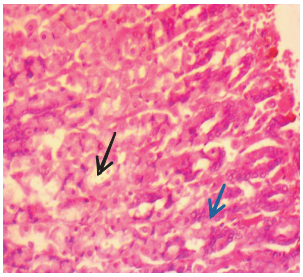

Fig.3a Control; Mucous cells (black arrow) Parietal cells (blue arrow). H&E x400

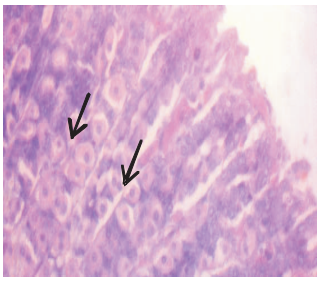

Fig.3b Stomach, showing hyperplastic mucous cells (top-arrows) and normal parietal cells (bottom-arrow) in rats treated with 5% alcohol and omega-3. H&E x400

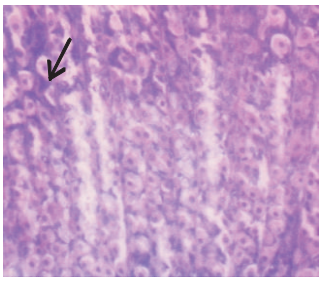

Fig.3c Stomach – showing atrophy of parietal cells (arrows) in rats treated with 5% alcohol alone. H&E x400

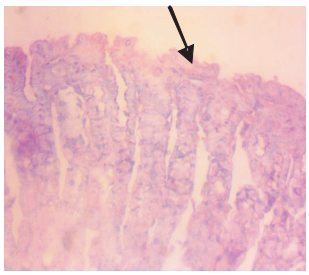

Fig.3d Stomach- showing hyperplasia of mucous cells (arrow) of rats treated with 20% alcohol and omega-3. H&E x400

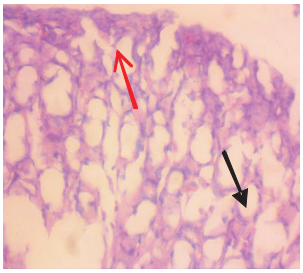

Fig.3e stomach- showing necrosis of mucous (red arrow) and parietal cells (black arrow) of rats treated with 20% alcohol alone. H&E x400

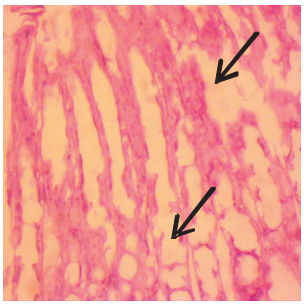

Fig.3f Stomach- showing diffuse necrosis of fundic cells (arrow) of rats treated with 40% alcohol alone. H&E x400

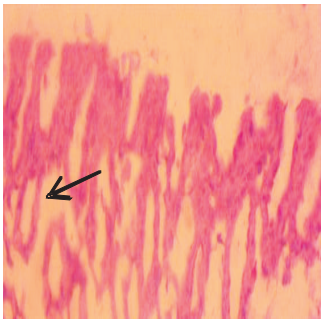

Fig.3g Stomach- showing atrophy of mucous neck cells (arrow) of rats treated with 40% alcohol and omega-3. H&E x400

Effect of Alcohol Administration on Histo-architecture of the duodenum in Experimental Rats (Figs 4a -4g)

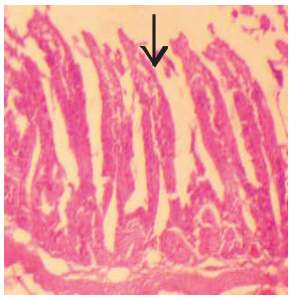

Fig.4a: Control Rats- Duodenum- showing tall villi (arrow). H&E x100

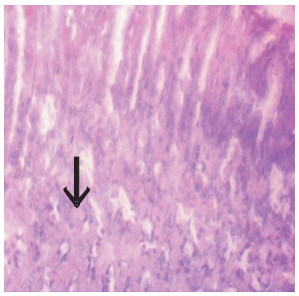

Fig.4b Duodenum- showing mild diffuse atrophy of brunner's glands (arrow) in rats treated with 5% alcohol and omega-3. H&E x100

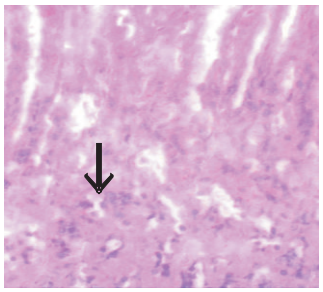

Fig.4c Duodenum- showing diffuse atrophy of Brunner's glands(arrows) in rats treated with 5% alcohol alone. H&E x100

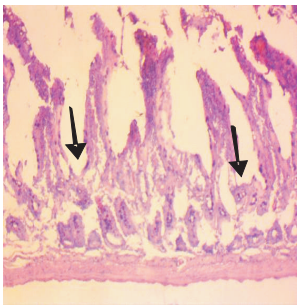

Fig.4d Duodenum- Showing necrosis of crypts and glandular cells in rats treated with 20% alcohol. H&E x100

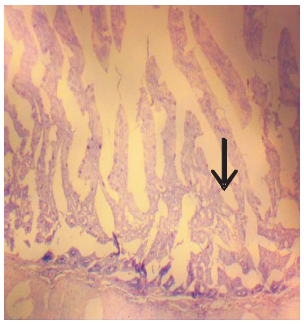

Fig.4e Duodenum- Showing mild resolution of the necrosis in rats treated with 20% alcohol and

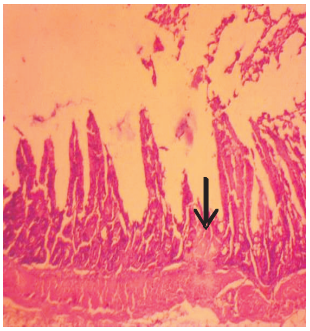

Fig.4f Duodenum showing mild diffuse atrophy of brunner's gland in rats treated with 40% alcohol and omega-3 H&E x100

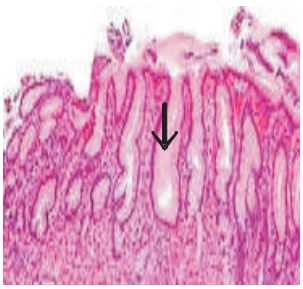

Fig.4g Duodenum showing congestion and diffuse atrophy treated with 40% alcohol H&E x100

alcohol+omega-3 treatment showed increased gastrin secretion compared with 0.05g/kg bw alcohol alone. 0.4g/kg bw alcohol+omega-3 treatment showed decreased gastrin secretion compared with 0.4g/kg bw alcohol alone

Secretin

The result showed an increase in serum secretin with increasing dose of alcohol (Fig. 2). Treatment with 0.2g/kg bw alcohol + omega and 0.4g/kg bw alcohol alone caused increased secretin level compared with control and omega alone. 0.2g/kg bw alcohol + omega treatment showed increased secretin level compared with 0.2g/kg bw alcohol alone. 0.4g/kg bw alcohol + omega treatment showed decreased secretin level compared with 0.4g/kg bw alcohol alone.

Histopathological studies

In the stomach with 0.05g/kg bw alcohol treatment, there were no marked alterations. There was atrophy and necrosis of the parietal and mucus cells with 0.2g/kg bw alcohol treatment and diffuse necrosis of these cells with 0.4g/kg bw alcohol treatment (Fig3a – Fig3g). In the duodenum, there was mild diffuse atrophy of the brunner's gland with 0.05g/kg bw alcohol treatment, necrosis of crypts and glandular cells with 0.2g/kg bw alcohol. This showed some resolution in the 0.2g/kg bw alcohol+omega-3 group. In the 0.4g/kg bw alcohol group, there was diffuse atrophy of the brunner's gland, there was no resolution seen with treatment with omega-3 in the 0.4g/kg bw alcohol+omega-3 group (Fig4a – Fig4g).

Discussion

Alcohol beverages with low ethanol content like beer and wine are strong stimulant of gastrin release. Whereas beverages with high ethanol content like whisky and gin do not stimulate gastric acid secretion or release of gastrin.[7] Mild to moderate consumption of low dose alcohol could possess health benefits,[8] however when consumed in high concentration especially over a long time, it could result in multiple pathologies.[9] High doses, greater 10% has been associated with mucosal damage.[10] These observation and many other reported alcohol induced pathologies have not discouraged the heavy consumption of alcoholic beverages globally.[11]Documented literature shows that alcohol interferes with gastrin and secretin secretion on a concentration dependent manner.

Results from this study reveal a dose-dependent increase in gastrin levels, with 40% alcohol group having the highest level of gastrin secretion when compared with control. This is in line with the findings of Vera et al,[12] who also observed an increase in gastrin level with increasing alcohol dosage. Beverages with higher ethanol content such as whisky, gin, or vodka may actually inhibit gastric acid secretion or release of gastrin[7].in this study, co-administration of omega-3 to alcohol treated rats did not improved serum gastrin level in high dose alcohol (40%). The mechanism behind the action of Omega-3 in causing decrease in gastrin is unclear but may be attributed to the action of Omega-3 in ameliorating the damage to the gastric parietal cells caused by high dose alcohol. Therefore, it is possible that Omega-3 prevents back diffusion of H^+ ions secondary to disruption of the mucosal barrier and thus plays a part in stimulating gastric acid secretion. Also, release of gastric acid inhibitors such as somatostatinwhich mediate the inhibitory effect on the parietal cells by binding to parietal cells via G-coupled receptor to reduce secretion may be blocked by Omega-3 which will ultimately cause decrease in gastrin secretion. Lianus et al,[13] reported that in humans plasma secretion level increases significantly from a basal of 1.21±14 to 1.64±24pg/ml at 60mins after oral ingestion of alcohol. Alcohol increases the emptying of acidic chyme into the duodenum stimulating the release of secretin by the S-cells of the duodenum. Result from figure 2 shows an increase in secretin level with increasing concentration of alcohol, and in combination with Omega-3, showing similar increase in secretion like gastrin with increasing concentrations (5%, 20% and 40%). This is in congruent with the findings of Nishiwakiet al.[14] who reported that alcohol stimulates release of endogenous secretin and pancreatic secretion by increasing duodenal acid load from the stomach. However, secretin has an inverse action to gastrin, as it inhibits gastric secretion and motility but stimulates increased bicarbonate rich pancreatic juice, which is important for digestion.

Alcohol consumption has been associated with assault on the cellular integrity of the pancreas and is an independent risk factor for the development of pancreatitis.[15] Alcohol consumption especially in high doses can result in inflammation of the gastric mucosa. According to Chi- chang et al,[16] a reduction in gastric mucous has been implicated in alcohol-induced gastric ulcers. Azzumet al.[17] in their study reported that an optimal gastric mucus secretion remains a vital factor protecting the gastric mucosa.

From the histological slides, reduced small intestinal enterocytes could also be responsible for the decrease in the enterocytes turnover in the small intestine. This could also be the cause for the impaired absorptive function.[18] Result from the histologic studies reveal that alcohol poses histopathological threat to soft tissues, causing excoriation of stomach and intestine. These account for the altered secretory functions of the GIT. Omega-3 administration to alcohol treated rats showed potency for the improvement/amelioration of soft tissue damage such as the pancreatic tissue as

suggested by,[19]however, this was not clearly seen in this study.

Conclusion

Persistent ingestion of alcohol over time especially highly concentrated forms (gin, whisky vodka), can distort the mucosa and submucosa of the stomach and duodenum, resulting amongst other symptoms in the elevation of serum levels of gastrin and secretin. Gastrin is essential in the digestion of meat protein while secretin is needed for regulation of pancreatic secretions especially bicarbonate. Elevation in serum gastrin levels can trigger abdominal pain, diarrhea, decrease appetite and unintended weight loss due to poor nutritional digestion and absorption and these deleterious actions of alcohol are often not ameliorated by use of anti-oxidants Such as Omega-3. The findings from this study will help shield some light on the reason for malnourishment seen with long-term consumption of concentrated alcohol.

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