Evaluation of Long Term Alcohol Consumption on Gastric Acid Secretion and the Histomorphometry of the Stomach in Adult Male Wistar Rats

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Abstract

This study was aimed at investigating to check the effects of long term consumption of varying concentrations of alcohol on gastric acid secretions and the histomorphometry of the stomach of adult male Wistar rats, and the possible ameliorative effect of omega-3 fish oil. Eighty (80) adult male Wistar rats weighing 180-230 gm were used. The rats were divided into eight groups containing ten (10) rats each group. Group I served as the control; Groups II, III and IV were given 5%, 20% and 40% alcohol respectively; Groups V, VI and VII were given 5% alcohol+Omega-3 fish oil; 20% alcohol+Omega-3 fish oil and 40% alcohol+Omega-3 fish oil, respectively. The Group VIII was administered Omega-3 fish oil alone. The alcohol was given at a dose of 0.005 ml/g body weight once daily using an orogastric canular. The Omega-3 fish oil was given at a dose of 0.2 ml/g body weight. Alcohol administration lasted for twelve weeks, at the end of which the rats were sacrificed by cervical dislocation. Gastric secretions were estimated, and tissues samples from the stomach collected for histomorphometric studies. The results showed that 20% alcohol caused a significant increase in gastric acid secretion when compared with the control and omega-3 only group. 40% and omega-3 group caused a decrease in gastric acid secretion when compared with the control. The histomorphometry of the stomach revealed a decrease in parietal cell and mucous cell count with increasing alcohol concentrations. Omega-3 administration showed only mild amelioration to these digestive alterations.

1 Introduction

A large amount of evidence from longitudinal epidemiological studies suggests that people consuming light- to- moderate amounts of alcohol have a lower risk of dementia¹, or better cognitive function² and lower risk of ischemic stroke³, than do persons who either abstains from or consume heavy amounts of alcohol. It is currently unclear which biological mechanisms could be involved in these associations.

Alcohol in the form of ethanol is probably the commonest ‘recreational drug’ in Western societies. About 90% of the population have been reported to consume alcohol from time to time, while 30% ends up developing alcohol related disorders⁴. Moderate Alcohol intake however has been reported to have health benefits⁵. Problems arise when it is abused. Alcohol dependence (alcoholism) in male (10%) and in female (3–5%) has been documented⁶. Other studies have observed other patterns in alcohol injeiction among the population, with increase intake of alcohol amount pregnancy women and young people⁷.

Ethanol is the most common recreational alcohol consumed and it has also been found to have useful as a preservative (diet and medicinal), antiseptic (hygienic). The LD₅₀ of ethanol in rats is 10.3 g/kg⁷.

Alcohol has associated with systemic disorders amongst which include tumors in the gastrointestinal tract⁸, reduced kidney...
function\textsuperscript{15}, confusion, ataxia and loss of social inhibition due to cerebellar atrophy\textsuperscript{11} and cardiomyopathy\textsuperscript{12,13}.

1.1 Alcohol Metabolism

The breakdown of alcohol by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) is the most common pathway for alcohol metabolism\textsuperscript{14}. The first step involves metabolism of alcohol, by ADH to a highly toxic substance called acetaldehyde. Second step, acetaldehyde is then metabolized to acetate, which is then broken down into carbon dioxide and water for easy elimination. Alcohol can also be metabolized to yield the byproduct acetaldehyde by enzymes cytochrome P450 2E1 (CYP2E1) and catalase\textsuperscript{14}.

Differences in alcohol metabolism may put some people at greater risk for alcohol problems\textsuperscript{14}. A study by Yin et al.\textsuperscript{15}, compared the isoenzymes of ADH and ALDH from surgically-excised esophageal and gastric mucosa and reported the following. There was increase activity of esophageal ADH was elevated by approximately 4-fold and the activity of ALDH accounted for 20% of the stomach enzymes, suggesting that an accumulation of intracellular acetaldehyde exists during ethanol ingestion. This toxic and reactive metabolite may be involved in the mechanism of ethanol-associated esophageal disorders. And according to Ajeigbe et al.\textsuperscript{16} a decrease in the activity of the mucosal disaccharides and an increase in permeability that facilitates the output of water and solutes (Sodium and Chloride) into the intestinal lumen.

To this effect, the study evaluated the effects of long term consumption of varying concentrations of alcohol (5%, 20% and 40%) on gastric acid secretions, as well as the histomorphometry of stomach.

2 Material and Methods

2.1 Materials

Langerdoff apparatus, syringes, dissecting sets, sample bottles, cotton wool, glass slides and slide rack, sensitive electronic weighing balance, micropipettes (REMI) 100-1000µl.

2.2 Drugs and chemicals

Analytical grade alcohol, phenolphthalein salt, Omega 3 Fish Oil supplements from BR Pharmaceuticals,10% Formal Saline, sodium hydroxide, paraffin, xylene, Haematoxylin-Eosin Stain.

2.3 Reconstitution of the alcohol

The various concentrations of alcohol were reconstituted as follows: 5% alcohol concentration was derived from 5 ml absolute alcohol mixed with 95 ml of distilled water; 20% alcohol concentration was derived from 20 ml absolute alcohol mixed with 80ml of distilled water; 40% alcohol concentration was derived from 40 ml absolute alcohol mixed with 60 ml of distilled water.

2.4 Animals

Eighty (80) male albino rats of the Wistar strain weighing between 180 gm – 230 gm were used for this study. The rats were procured from the animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka. They were housed in standard wooden cages, under natural 12-hour light and 12 hour darkness, and fed with commercial chow. They were given water ad-libitum. The animals were handled according to the guidelines for the care and use of Laboratory Animals by the Research Ethics Committee of Delta State University, Abraka.

2.5 Experimental design

Based on related body weights, the rats were divided into eight (8) groups of ten (10) rats (n = 10) each. Three different concentrations of alcohol were used in this study: 5%, 20% and 40%. The rats were given single daily oral doses of the various concentrations of alcohol, at a dose of 0.005ml/g body weight with the aid of an oro-gastric cannula. Rats were also given Omega-3 fish oil daily with the aid of an orogastric cannula at a dose of 0.01ml/g body weight. The treatment groups are as follows: Group 1: rats were given only clean drinking water and normal feed ad libitum; Group 2: rats were given 5% alcohol only; Group 3: rats were given 20% alcohol only; Group 4: rats were given 40% alcohol only; Group 5: rats were given 5% alcohol and omega-3 fish oil only; Group 6: rats were given 20% alcohol and omega-3 fish oil only; Group 7: rats were given 40% alcohol and omega-3 fish oil only and Group 8: rats were given omega-3 fish oil only. The duration of the treatment was for twelve weeks.

2.6 Measurement of gastric acid secretion

2.6.1 Method

(Continuous perfusion method by Ghosh and Schild\textsuperscript{17}, modified by Amure et al., (1964)). Five rats from each group were fasted overnight prior to the experiment, allowing water only. The weight of the rats were taken with a weighing balance. The rats were anaesthetized using sodium pentobarbital at a dose of 15 mg/kg body weight. After the anaesthesia took effects, the limbs of the rats were tied to a dissecting board. Tracheostomy was carried out to aid breathing. The esophagus, lying posterior to the trachea was located and a cannula was passed from the Langerdoff’s apparatus, into the mouth through the esophagus to the stomach and tied with a thread to prevent gastrointestinal reflux of gastric content. Laparotomy was carried out to access the stomach. The pyloric end of the stomach was located and an incision was made at its superior end. A rubber tube was inserted through this incision into the stomach and tied with a thread. The contents of the stomach were flushed out by allowing the normal saline heated to 37°C at the Langerdoff’s apparatus to run into the stomach and out through the rubber tube. The animals were allowed to rest for 10 minutes and
thereafter, the normal saline running from the Langerdoff’s apparatus was regulated at an adjustable knob to collect at least 10ml in 10minutes of the effluent from the stomach in a beaker. The acidity of each ten minutes effluent collected was assayed by titration. Sodium hydroxide (base) was filled in 50 ml burette. The NaOH was titrated against 10ml of the gastric effluent in a beaker after adding 2 drops of phenolphthalein as an indicator under a white tile. The colour change (pink) was noted and the volume of NaOH used on the burette graduation was then recorded for each titration. This process was repeated for another eight titrations.

2.6.2 Sample collection and determination of gastric parietal cell mass and mucous cell population

At the end of the experiment, five rats from each group were collected and weighed. They were fasted overnight and sacrificed by cervical dislocation. The stomach was removed as quickly as possible into normal saline, opened along the greater curvature, washed and transferred into a beaker containing 10% formalin. Sections were prepared from strips removed from the fundic area of the stomach and stained using the method of Oluwole et al.19, using the Haematoxylin and Eosin stained. The various gastric mucosal secretory cells were clearly differentiated, taking up different colours. The nuclei of the parietal cells were stained deep blue while the mucous cells were clearly vacuolated. Parietal cell mass index was calculated as described by Perraso et al.20, as the number of cells per mm² multiplied by the thickness of the glandular layer. Five counts from randomly selected fields were made on each section, and the average count per unit area was calculated for each stomach by dividing the number of cells seen by the number of counts made21,22.

2.7 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. P-values of less than 0.05 were considered statistically significant.

3 Results

3.1 Effect of alcohol administration on the level of gastric acid secretion

The effect of alcohol administration on the level of gastric acid secretion in experimental rats is shown in fig 1. 20% alcohol caused a significant increase in gastric acid secretion when compared with control and omega alone groups. 40% + omega caused a decrease in gastric acid secretion when compared with control.

Fig 1: Showing the effects of alcohol on the level of gastric acid secretion in experimental rats expressed as Mean±SEM

Ω: Omega-3, ALC: Alcohol, (a) Significant increase when compared to control, (b) significant decrease when compared to control, (+) significant increase when compared with omega-3, (−) significant decrease when compared with omega-3, (*) significant increase when alcohol alone is compared with alcohol+omega-3, (#) significant decrease when alcohol alone is compared with alcohol+omega-3

3.2 Effect of alcohol administration on gastric mucous cell count

The effect of alcohol administration on Gastric mucous cell count in experimental rats is shown in fig 2. There was a general decrease in the gastric mucous cell count in all groups except those treated with 5% alcohol +omega when compared with the control group and omega alone groups. Gastric mucous cell count decreased with increasing alcohol concentration.

3.3 Effect of alcohol on parietal cell count

The effect of alcohol administration on parietal cell count in experimental rats is shown in fig 3 there was a general decrease in the parietal cell count in all groups except those treated with 5% alcohol + omega when compared with the control group 20%, 40% alcohol alone and 40% alcohol + omega showed decreased parietal cell count compared omega
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alone group. Parietal cell count decreased with increasing alcohol concentration.

Fig 2: Showing the effects of alcohol on the gastric mucous cell count of experimental rats expressed as Mean±SEM

Ω: Omega-3, ALC: Alcohol, (a) Significant increase when compared to control, (b) significant decrease when compared to control, (+) significant increase when compared with omega-3, (—) significant decrease when compared with omega-3, (*) significant increase when alcohol alone is compared with alcohol+omega-3, (#) significant decrease when alcohol alone is compared with alcohol+omega-3.

Fig 3: Showing the effects of alcohol on the parietal cell count of experimental rats expressed as Mean±SEM

Ω: Omega-3, ALC: Alcohol, (a) Significant increase when compared to control, (b) significant decrease when compared to control, (+) significant increase when compared with omega-3, (—) significant decrease when compared with omega-3, (*) significant increase when alcohol alone is compared with alcohol+omega-3, (#) significant decrease when alcohol alone is compared with alcohol+omega-3.

4 Discussion

Nutrition as a process has two important functions: to provide energy and maintain body functions and structure. Food supplies energy, as well as providing the building blocks needed to replace worn or damaged body cells. It also provides the nutritional components required for body functions. The gastrointestinal (GI) system is essentially for this purpose, and any impairment to its function would result in deleterious effects arising from inappropriate food metabolism and energy supply to the cells. Alcohol consumption has made up an important topic in several studies, with debates centered on its nutritional benefits as well as its potential hazard to the body and specifically the gastrointestinal system. Alcohol is a low molecular weight, readily diffusible compound that can be absorbed much more slowly from the stomach than the small intestine. Alcohol can also be oxidized in the gastrointestinal tract (GIT), where it undergoes first-pass metabolism and influences gastrointestinal motility, secretions and enzyme activities. Alcohol consumed in small quantities or low concentrations has mild effects on the body system, chronic consumption of alcohol has been associated with multiple pathologies, and a concentration above 10% has been observed to produced mucosal damage. Despite these effects on the body, there is still an increase in the consumption of high grade alcoholic beverages.
Omega-3 fatty acids are believed to provide a wide range of health benefits. They are very useful in inflammatory regulations. Upon consumption, Omega is taken into the cell membranes. It reduces the arachidonic acid that is used as a substrate for the synthesis of proinflammatory eicosanoids. It has become a popular supplement hence, its use in this study to evaluate its nutritional benefits in chronic alcoholics and its potency in protecting against or ameliorating the toxic effects of alcohol metabolism in the body.

In the stomach, the chemical degradation of the food continues with the help of gastric acid and various digestive enzymes such as rennin, pepsin and lipase. An in balance in gastric acid secretion and mucosa secretion has been documented as a causing of gastric ulceration. Gastric acid secretion have been reported to effect by alcohol in respective of the route of administration and there is varies in this secretory response in the stomach, however, depending on the species studied and the alcohol concentrations used. Administration of alcohol in this study did not affect gastric acid secretion in all concentrations. This is in line with the findings of Char et al. who reported a mild stimulation of gastric acid secretion with low dose alcohol injection and inhibition of gastric acid secretion with high dose. Omega administration with alcohol reduced gastric acid secretion in low concentration (5%) alcohol. This suggests that omega-3 may protect against gastric irritation and ulcer in cases of excessive secretion of gastric acid by lower concentration alcohol as contained in beer.

The parietal cells are responsible in initiating digestion through the process of acid secretion. The parietal cell count decreased in all the groups, except in the 5% alcohol + omega group. These results are similar to that of Zarebska et al. Alcohol consumption can cause inflammation of the gastric mucosa. According to Chi-chang et al., a reduction in gastric mucous has been implicated in alcohol-induced gastric ulcers. Hence, the mucous cell counts. From this study, a general decrease was observed in all the treatment groups, and is in line with studies done by Oluwol et al. A reduction in the mucous cell count will lead to reduction gastric mucus, and according to Chi-chang et al., is implicated in alcohol-induced gastric ulcers. Also, according to Azzum et al., an increase in the gastric mucus secretion remains the main factor protecting the gastric mucosa.

5 Conclusion

Alcohol consumption in its respective concentrations cause alterations in the gastrointestinal micro architecture posing a risk of GI ulcers via mucosal excoriations and cellular necrosis. This effect was manifested in this study to be concentration dependent, with higher concentration causing more anatomical distortions to the gastrointestinal epithelium. These changes in the micro anatomy of the gastrointestinal epithelium correlate with the alterations observed in the gastrointestinal motility and secretory functions. It attempts to ameliorate these effects with omega-3 may only be beneficial with respect to the concentrations of alcohol consumed.

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7 Conflict of interest statement

Authors have declared that no competing interests exist.

8 Author’s contributions

ECA and AEO carried out literature review and draft the manuscript. ECA designed and monitored the experimental protocol. OE, LOE, TMED and CUO collected the material and performed whole experimental procedures. All authors read and approved the final manuscript.

9 References


