EFFECTS OF MELATONIN ON ALCOHOL INDUCED STOMACH TOXICITY IN ADULT WISTAR RATS

BY

OBATA-GODWIN OSELENE NORRIS

BMS2101342

A PROJECT SUBMITTED TO THE DEPARTMENT OF ANATOMY,

SCHOOL OF BASIC MEDICAL SCIENCES,

UNIVERSITY OF BENIN, BENIN CITY, EDO STATE,

NIGERIA

SUPERVISED BY:

DR. EDOBOR OBAYUWANA

NOVEMBER 2025

CERTIFICATION

This is to certify that this project titled '**Investigating the Effects of Melatonin on Alcohol-Induced Gastric Ulcer in Adult Male Wistar Rats**.' would be carried out by Obata-Godwin Oseleme Norris with matriculation numbetr number; BMS2101342 in the Department of Anatomy, College of Basic Medical Sciences, University of Benin.

NAME OF PROJECT SUPERVISOR: ----------

SIGNATURE: ----------

DATE: ----------

NAME OF EXTERNAL EXAMINER: ----------

SIGNATURE: ----------

DATE: ----------

NAME OF HEAD OF DEPARTMENT: ----------

SIGNATURE: ----------

DATE: ----------

DEDICATION

This work is dedicated to the Almighty God, the Ever-loving One who has kept me alive throughout my academic journey and the duration of this research, To my parents whom has always been there for me

ACKNOWLEDGEMENT

First and foremost, I would like to express profound gratitude to God Almighty, Adonai El Roi, whose unfailing grace, wisdom, and steadfast presence have been the foundation and strength throughout this academic journey. Every success recorded in the course of this work is a reflection of divine guidance and mercy.

Sincere appreciation is expressed to the project supervisor Dr Obaywana Edobor for invaluable insights, scholarly guidance, and motherly patience. The academic direction, constructive feedback, and freedom granted to explore this research area were instrumental in shaping and refining this research.

My heartfelt gratitude is also given to my parents, Mr. Obata Godwin and Mrs. Obata Grace, whose love, prayers, and unwavering support, both financial and emotional, have been central to the completion of this work. I also extend my deepest appreciation equally to Mr. Amandi Ifeanyi and Mr. Wisdom Eromosele for their encouragement, understanding, and steadfast belief in this academic pursuit.

To my friends, Okuna Areghe Phoebe, Maduike Marvin, Iselobor Genesis, Henry, Francis, Oshioke, Raphael and my other friends, I cannot fully express the magnitude of what your support has meant to my academic journey; your friendship, advice and encouragement have continually bowed my heart in gratitude before Almighty God.

TABLE OF CONTENTS

ABSTRACT

Alcohol (ethanol) is a widely consumed psychoactive substance known to induce oxidative stress and gastrointestinal mucosal damage, particularly in the stomach. Chronic alcohol exposure generates reactive oxygen species (ROS) and disrupts mucosal integrity. M melatonin on alcohol-induced gastric toxicity in adult male Wistar rats. Forty adult male Wistar rats (150–180 g) were randomly divided into four groups (n=10): control, melatonin only (5 mg/kg), alcohol only and melatonin plus alcohol. All treatments were administered orally via gavage for 28 days. After the exposure period, a weight test was done, and gastric tissues were harvested for histopathological analysis. Statistical analysis was performed using one-way ANOVA with significance set at $p<0.05$. Alcohol induced ulceration in the mucosa of the stomach, and the ulcer induced was irregularly -shaped. The control group showed a normal gastric architecture, while the group given alcohol only exhibited disruption of the muscularis mucosa with the formation of a irregular-shaped ulcer. The group given alcohol and melatonin showed that melatonin attenuated the ulcerative lesion in the stomach, indicating that melatonin effectively resolved alcohol-induced gastric injury.

CHAPTHER ONE

INTRODCTION

1.1. BACKGROUND OF STUDY

Alcohol is widely consumed globally and is known to significantly impact multiple organ systems, including the liver, nervous system, kidneys, and gastrointestinal tract (Nutt et al., 2021; Obayuwana et al., 2024). Ethanol metabolism in the liver via enzymes such as alcohol dehydrogenase, microsomal ethanol-oxidizing system and catalase, produces reactive oxygen species (ROS) that contribute to oxidative stress (Obayuwana and Okereke 2024). Although much attention has been given to alcohol's hepatic toxicity, studies have also shown that chronic alcohol consumption can damage the kidneys and stomach through oxidative mechanisms.

Melatonin, an endogenous hormone synthesized in the pineal gland, gastrointestinal tract, and other tissues (Hardeland, 2017), has demonstrated protective effects on the gastrointestinal mucosa (Konturek et al., 2007). Its anti-inflammatory, anti-secretory, and mucosal barrier-stabilizing properties (Nabavi et al., 2019) suggest a possible role in modulating the gastric environment and potentially inhibiting H. pylori colonization. However, limited studies have explored melatonin's effect on alcohol-induced susceptibility to H. pylori infection.

1.2 STATEMENT OF RESAERCH PROBLEM

Alcohol (ethanol) is a widely consumed psychoactive substance that poses significant public health concerns due to its capacity to induce systemic toxicity when misused (Tedor, 2021). One of the lesser-explored but clinically relevant consequences of chronic alcohol intake is its detrimental effect on the gastrointestinal tract, particularly the duodenum. Upon ingestion,

alcohol is rapidly absorbed in the gastrointestinal system, where it generates reactive oxygen species (ROS) and other free radicals as metabolic by-products, primarily through its conversion to acetaldehyde (Na and Lee, 2017). These reactive intermediates compromise the mucosal barrier, provoke inflammatory responses, and induce oxidative damage in intestinal tissues.

Despite extensive studies on hepatic and neurological toxicity of alcohol (Nutt *et al*., 2021; Obayuwana and Okereke, 2024), research on its impact on small intestinal structures such as the duodenum remains limited. This is concerning, given the critical role of the duodenum in digestion, nutrient absorption, and its close proximity to the initial site of alcohol absorption. The structural and functional impairment of the duodenum due to alcohol exposure can disrupt gut homeostasis and contribute to gastrointestinal disorders, yet it remains under-investigated in toxicological studies.

Therefore, this study is designed to address the gap in knowledge regarding the histopathological and biochemical alterations caused by chronic alcohol exposure in the duodenum. By focusing on alcohol as the primary toxicant, this research aims to highlight the mechanisms by which it induces oxidative stress and tissue damage in the small intestine, thereby providing a foundation for future studies on protective interventions.

1.3 JUSTIFICATION OF STUDY

Alcohol-induced gastric damage is a prevalent contributor to upper gastrointestinal disorders. Chronic alcohol exposure can increase susceptibility to H. pylori colonization due to its damaging effects on the mucosa. Understanding how melatonin may counteract alcohol-induced gastric injury could provide novel insights for gastroprotective therapies.

1.4. AIM AND OBJECTIVE OF STUDY

1.4.1. AIM OF STUDY

To investigate the effects of melatonin on alcohol-induced gastric toxicity in adult male Wistar rats.

1.4.2. OBJECTIVES OF STUDY

1. To determine the effects of alcohol on the weight of adult Wistar rats.
2. To determine the effects of melatonin on the weight of adult male wistar rats
3. To examine the histological impact of alcohol on the stomach

CHAPTER TWO

LITERATURE REVIEW

2.1 MELATONIN

2.1.1 Introduction

Melatonin or 5 methoxy-N-acetyltryptamine was discovered and isolated from bovine pineal in 1958 by Aaron Lerner (Lerner *et al*., 1958). Melatonin is the main hormone secreted by the pineal gland. Extrapineal sources of melatonin were reported in the retina, bone marrow cells, platelets, skin, lymphocytes, Harderian gland, cerebellum, and especially in the gastrointestinal tract of vertebrate species. Indeed, melatonin is present but can also be synthesized in the enterochromaffin cells; the release of gastrointestinal melatonin into the circulation seems to follow the periodicity of food intake, particularly tryptophan intake. It is noteworthy that the concentration of melatonin in the gastrointestinal tract surpasses blood levels by 10-100 times and there is at least 400 times more melatonin in the gastrointestinal tract than in the pineal gland (Bubenik, 2002). Melatonin in the gastrointestinal tract of newborn and infant mammals is of maternal origin given that melatonin penetrates easily the placenta and is after secreted into the mother's milk. It has even been suggested that melatonin is involved in the production of mekonium (Bubenik, 2002). Melatonin in human breast milk follows a circadian rhythm in both preterm and term milk, with high levels during the night and undetectable levels during the day [14, 15]. No correlation was found between gestational age and concentration of melatonin. It is noteworthy that bottle milk composition does not contain melatonin in powder formula. Also, human colostrum, during the first 4 or 5 days after birth, contains immune – competent cells

(colostral mononuclear cells) which are able to synthesize melatonin in an autocrine manner (Pires-Lapa *et al*., 2013).

2.1.2 Dietary Sources of Melatonin

In recent decades, MLT has been detected in animal foods and edible plants due to its powerful effects on health, with concentrations varying markedly among different foods, levels can also change during food preparation (Carrillo-Vico *et al*., 2004). The content of MLT in food has also been shown to vary widely between species, with considerable differences in MLT concentrations being reported for a variety of food species and organs, ranging from pg/g to mg/g. It has also been established that MLT-rich foods may influence human health via an increase in MLT serum levels and antioxidant capacity. MLT can be distributed unevenly among individual animals and plants due to the different biophysical dynamic features of individual organs (Di *et al*., 1997).

2.1.2.1 Animal foods

Tan and his colleagues reported, for the first time, that MLT can be found in certain types of meat, including chicken, lamb, beef, and fish. TRP is an essential amino acid for humans. Since humans cannot produce TRP sufficiently, this amino acid must be absorbed from protein-containing foods through the small intestine. MLT is produced by metabolizing TRP via the serotonin pathway, and the BBB must be crossed in order to transport TRP into the brain for MLT synthesis. MLT levels are higher in eggs and fish compared to meat; in particular, MLT levels in solid dried eggs are 6.1 ± 0.95 ng/g, whereas those in salmon are 3.7 ± 0.21 ng/g (Di *et al*., 1997).

2.1.2.2 Human milk

Reppert and colleagues (1993) studied MLT for its potential physiological role in human milk, demonstrating that it could be transmitted into neonates through human milk by measuring human milk (Zawilska *et al*., 2009). The gastrointestinal tract of newborns and infant mammals contains MLT from the mother, since it transmits efficiently across the placenta and is absorbed from the mother's milk. MLT concentrations follow a circadian pattern in preterm and term breast milk, with peaks occurring at night and declining during the day (Ekmekcioglu, 2006).

2.1.2.3 Plant foods

MLT has been found in many commonly consumed fruits. The fruits most commonly studied for their MLT content are grapes, cherries, and strawberries, demonstrating differences in their cultivars (Ebisawa *et al*., 1994). MLT has also been detected in many plant-derived foods, including nuts, fruits, seeds, cereals, oils, and coffee beans. Many vegetables contain MLT, but the level is undetectable in potatoes and low in beets. The most studied vegetables are tomatoes and peppers, which contain relatively high levels of MLT within the vegetable group. Recently, MLT also was found in a variety of legumes, which are essential sources of protein, vitamins, and minerals. Recent research has focused on the benefits of these foods with regard to disease prevention, due to their fiber content, slow digestion of starch, prebiotic oligosaccharides, and phenolic content (Zawilska *et al*., 2009). Some of these compounds also possess antioxidant properties that may contribute to their potential health benefits in preventing diseases caused by oxidative stress (Zawilska *et al*., 2009).

2.1.3 Synthesis and Secretion

Melatonin is mainly synthesized by the pinealocytes from the amino acid tryptophan, which is hydroxylated (by the tryptophan-5-hydroxylase) in 5-hydroxytryptophan, then decarboxylated (by the 5-hydroxytryptophan decarboxylase) in serotonin. Two enzymes, found mainly in the pineal gland, transform serotonin to melatonin (Coon *et al*., 1995): serotonin is first acetylated to form N-acetylserotonin by arylalkylamine-N-acetyltransferase (AA-NAT, also called "Timezyme", is the rate-limiting enzyme for melatonin synthesis), and then N-acetylserotonin is methylated by acetylserotonin-O-methyltransferase (ASMT, also called hydroxyindole-O-methyltransferase or HIOMT) to form melatonin. Both AA-NAT and ASMT activities are controlled by noradrenergic and neuropeptidergic projections to the pineal gland (Simonneaux and Ribelayga, 2003). Norepinephrine, also called noradrenaline, activates adenylate cyclase which in turn promotes the melatonin biosynthesis enzymes, especially AA-NAT. Once synthesized, melatonin is quickly released into the systemic circulation to reach central and peripheral target tissues. Melatonin synthesis and secretion is enhanced by darkness and inhibited by light. Luminous information is transmitted from the retina to pineal gland through the suprachiasmatic nucleus (SCN) of the hypothalamus. In humans, its secretion starts soon after sundown, reaches a peak in the middle of the night (between 2 and 4 in the morning) and decreases gradually during the second half of the night. Nearly 80% of the melatonin is synthesized at night, with serum concentrations varying between 80 and 120 pg/ml. During daylight hours, serum concentrations are low (10-20 pg/ml) (Karasek and Winczyk, 2006). Serum concentrations of melatonin vary considerably with age, and infants secrete very low levels of melatonin before 3 months of age. Melatonin secretion increases and becomes circadian along with child development: Sadeh (1997) reported an association between melatonin secretion

and organization of sleep-wake rhythm from 6 months of age. However, more recent studies suggest that melatonin rhythm is set around 3 months of age in typical development, at the same time that infants begin to have more regular sleep–wake cycles associated with nighttime sleep lasting 6-8 h (Joseph *et al*., 2014). In 3-years-old children, a stabilization of the sleep-wake rhythm is observed, which corresponds to a regular melatonin secretion rhythm. Nocturnal concentration peaks are the highest between the 4 and 7 years of age, and then decline progressively.

2.1.4 Pharmacokinetics

After intravenous administration, melatonin is rapidly distributed (distribution half-life of 0.5 to 5.6 minutes) and eliminated. After oral administration, plasma concentration peak arises within 60 minutes. Plasma concentrations diminution is biphasic, with a half-life of respectively 2 and 20 minutes (Claustrat *et al*., 2005). Intake of an usual dose (i.e., 1 to 5 mg), allows within the hour after ingestion, melatonin concentrations 10 to 100 times higher than the physiological nocturnal peak to be obtained, with a return to basal concentrations in 4 to 8 hours. A bioavailability study in four male healthy volunteers showed a plasma melatonin peak varying between 2 and 395 nmol/L and an elimination half-life of 47± 3 min (mean ± SD) after oral administration of a 500 µg dose. Bioavailability varied from 10 to 56% (mean 33%). After intravenous or oral administration, melatonin is quickly metabolized, mainly in the liver and secondarily in the kidney. However, after intravenous administration, the hepatic bio-degradation is less important due to the absence of hepatic first pass. It undergoes hydroxylation to 6-hydroxymelatonin by the action of the cytochrome P450 enzyme CYP1A2, followed by conjugation with sulfuric acid (90%) or glucuronic acid (10%) and is excreted in the urine. About 5% of serum melatonin is excreted unmetabolized also in urine. The principal metabolite,

the 6-sulfatoxy-melatonin (6-SM), is inactive, and its urinary excretion reflects melatonin plasma concentrations.

2.1.5 Mechanisms of Action

Melatonin acts through different molecular pathways. The best characterized pathway is the activation of two types of membrane specific receptors: high affinity ML1 sites and low affinity ML2 sites (Dubocovich, 1995). The activation of ML1 receptors, which are G protein-coupled receptors, leads to an inhibition of the adenylate cyclase in target cells. The activation of ML2 receptors, currently called MT3, leads to phospho-inositides hydrolysis. MT3 is expressed in various brain areas and has been shown to be the enzyme quinone reductase 2. Two sub-types of the ML1 receptor have been described Mel1a and Mel1b. Mel1a (or MT1) is encoded in human chromosome #4 (4q35.1) and consists of 351 amino acids. Mel1a is widely distributed in the pars tuberalis of the anterior pituitary and the SCN of the hypothalamus (which is the anatomic site of the circadian clock), and also in the cortex, thalamus, substantia nigra, nucleus accumbens, amygdala, hippocampus, cerebellum, cornea and retina (Jockers *et al*., 2008). Mel1b (or MT2) is encoded in human chromosome #11 (11q21-q22) and consists of 363 amino acids. Mel1b is distributed mainly in the retina and secondarily in the hippocampus, cortex, paraventricular nucleus, and cerebellum (Zawilska, 2009). Melatonin has also an intracellular action by binding, on the one hand, to cytosolic calmodulin, and on the other hand, to two receptors of the Z retinoid nuclear receptors family. Melatonin receptors have been found in several central but also in peripheral tissues, including heart and arteries, adrenal gland, kidney, lung, liver, gallbladder, small intestine, adipocytes, ovaries, uterus, breast, prostate, and skin (Ekmekcioglu, 2006).

2.1.6 Effects of Melatonin

2.1.6.1 Physiological Effects

Melatonin regulates circadian rhythms such as the sleep-wake rhythm, neuroendocrine rhythms or body temperature cycles through its action on MT1 and MT2 receptors (Ekmekcioglu, 2006). Ingestion of melatonin induces fatigue, sleepiness and a diminution of sleep latency. Disturbed circadian rhythms are associated with sleep disorders and impaired health. For example, children with multiple developmental, neuro-psychiatric and health difficulties often show melatonin deficiency. When circadian rhythms are restored, behavior, mood, development, intellectual function, health, and even seizure control may improve. It should be noted that according to several studies, circadian rhythms are important for typical (normal) neurodevelopment and their absence suppresses neurogenesis in animal models (Kong *et al*., 2008).

2.1.6.2 Melatonin in Disorders and Therapeutic Effects

Therapeutic effects of melatonin have been reported in several disorders such as certain tumors, cardiovascular diseases or psychiatric disorders.Indeed, oncostatic effects of melatonin have been reported in several tumors (breast cancer, ovarian and endometrial carcinoma, human uveal melanoma, prostate cancer, hepatomas, and intestinal tumors) (Blask *et al*., 2005). These oncostatic effects have been attributed to the antioxidative role of melatonin given that oxidative stress is involved in the initiation, promotion and progression of carcinogenesis. Also, decreased melatonin levels (measures of blood melatonin or urinary excretion of 6-SM) were reported in patients with cardiovascular diseases. Inversely, melatonin treatment reduces blood pressure in patients with hypertension. Concerning psychiatric disorders, secretion disturbances of the pineal gland have been described in child and adult psychiatry, with notably in most studies a decreased

nocturnal melatonin secretion observed in major depressive disorder, bipolar disorder, schizophrenia or autism spectrum disorder (Morera-Fumero and Abreu-Gonzalez, 2013).

2.1.6.3 Melatonin and Brain Protection

Neurological and neuropsychological disabilities caused by brain injuries are a major public health concern. Thus, reducing deficits after a stroke is a major issue. In this line, a number of recent studies have reported the important role of melatonin in neuroprotection in animal models of stroke (Kilic *et al*., 1999). Indeed, melatonin administration after an experimental stroke in animals reduces infarction volume. Such a protective effect can be seen in both gray and white matter and melatonin reduces also inflammatory response , cerebral oedema formation, and blood-brain barrier permeability (Chen *et al*., 2006). In addition, Kilic *et al*. (2008) investigated how sub-acute delivery of melatonin, starting at 24 hours after stroke onset, and continuing for 29 days can influences neuronal survival, endogenous neurogenesis, motor recovery and locomotor activity in mice submitted to an occlusion of the middle cerebral artery during 30 minutes. Furthermore, melatonin improved neuronal survival and enhanced neurogenesis, even when applied one day after stroke. In addition, the authors showed both motor as well as behavioral improvements after melatonin administration. Indeed, the results indicate that cell survival was associated with a long-lasting improvement of motor and coordination deficits as well as with attenuation of hyperactivity and anxiety of the animals as revealed in open field tests. Its neuroprotective activity in animal models of ischemic stroke, as well as its lack of serious toxicity suggests that melatonin could be used for human stroke treatment in the future.

2.2 ALCOHOL

The impact of excessive alcohol intake on human health is dramatic. Recent data show that alcohol consumption is responsible for approximately 6% of all deaths (3.3 million people) and 5.1% of the global disease burden.1 In addition to its addictive properties, alcohol consumption can lead to roughly 200 different diseases, including 14 different types of cancer (W.H.O, 2018). More than 50% of all alcohol-related deaths in both males and females are due to gastrointestinal diseases. The most common alcohol-associated causes of death are liver cirrhosis (50% of cases), pancreatitis (25%), and esophageal cancer (22%) (W.H.O, 2018). These data indicate that about 50% of deaths are due to end-stage cirrhosis, and approximately 500,000 annual deaths in Europe are related to alcohol misuse (W.H.O, 2018).

2.2.1 Effect of Alcohol Consumption on Organs

2.2.1.1 Liver

Alcohol is absorbed in the upper digestive tract (20% in the stomach and 75% in the first part of the small intestine), with the remaining 5% absorbed in the small intestine and colon.6,7 In fasting conditions, absorption is almost complete within one hour after intake: alcohol levels peak after 30–45 m and cannot be reliably measured 4–6 h after intake. If the alcoholic beverage is ingested with food (particularly fatty foods), the alcohol peak is delayed by 2–6 h.6,7 One endogenous factor that modulates ethanol absorption through the gastric mucosa is alcohol dehydrogenase (ADH). Approximately 10% of ingested ethanol is metabolized by gastric ADH, (Seitz *et al*., 1993) thus preventing it from entering systemic circulation (“first-pass metabolism [FPM]”). The intake of similar doses of ethanol (corrected for body weight) can lead women to have higher blood alcohol levels than men. This difference is due to both lower body water

content and reduced FPM (due to decreased gastric ADH activity) (Seitz *et al*., 1993). These factors increase ethanol bioavailability and contribute to the greater susceptibility of females to alcohol-related damage. Furthermore, anatomical changes (such as gastric resection) or histological modifications (e.g., metaplasia) of the antral parietal cells can affect alcohol bioavailability. Certain drugs (e.g., acetylsalicylic acid and proton pump inhibitors) can inhibit gastric ADH activity, particularly if low doses of alcohol are consumed (Seitz *et al*., 1993). Through the portal system, approximately 90% of absorbed alcohol reaches the liver, the primary site of its metabolism, where ethanol is metabolized to acetaldehyde via liver ADH.

2.2.1.2 Gastrointestinal tract

The digestive system is often one of the first to be affected by excessive alcohol consumption. The resulting damage can be either reversible or irreversible, depending on the frequency and mode of alcohol intake. From the oral cavity to the large intestine, alcohol can have profound toxic effects, resulting in various lesions throughout the gastrointestinal tract (Bujanda, 2000). Chronic alcohol consumption can lead to significant damage in the oral cavity. This includes stomatitis, often a result of nutritional deficiencies, and periodontal disease due to alterations in the oral microbiota. Changes in the bacterial environment can exacerbate gum disease and other oral health problems, highlighting the need for proper oral hygiene and dietary supplements for patients with chronic alcohol use. Alcohol can also cause substantial damage to the esophagus, both through direct toxicity to the mucosal lining and via alterations in esophageal motility and peristalsis (Bujanda, 2000). The pathophysiological mechanisms by which alcohol damages the epithelium include epithelial transport abnormalities, intercellular junction disruptions, and impairment of the mucosal barrier. Additionally, alcohol reduces lower esophageal sphincter pressure and affects both esophageal motility and gastric emptying (Haber and Kortt, 2021).

These changes increase the risk of developing gastroesophageal reflux disease, acute and chronic esophagitis, and Barrett's esophagus, a known precancerous condition. Excessive alcohol intake is also associated with a pro-emetic effect, which can lead to Mallory-Weiss syndrome, characterized by tears in the mucosal layer at the esophagogastric junction. Studies indicate that about 80% of patients with Mallory-Weiss syndrome had consumed large amounts of alcohol before the onset of symptoms (Haber and Kortt, 2021). Moreover, the stomach is also highly susceptible to damage from excessive alcohol consumption. The same mechanisms that cause esophageal damage can affect the stomach, leading to superficial gastritis, chronic atrophic gastritis, and, in the long term, intestinal metaplasia, a precursor to gastric cancer. Ethanol slows gastric emptying and increases gastric acid secretion, which can result in acute hemorrhagic gastritis. In addition to a wide array of symptoms, irritation and inflammation of the gastric mucosa are associated with an increased risk of peptic ulcer disease (Bujanda, 2000).

2.2.1.3 Pancreas

The relationship between alcohol consumption and pancreatic diseases has been a subject of considerable research and clinical interest (Yadav and Lowenfels, 2013). Chronic alcohol misuse is a well-established risk factor for the development of pancreatic diseases, ranging from acute to chronic pancreatitis and pancreatic cancer. In particular, pancreatitis is a common consequence of chronic alcohol consumption. It is estimated that approximately 70% of acute and 30% of chronic pancreatitis cases are attributable to alcohol misuse. As recently indicated by Rasineni et al., the pathophysiology of alcohol-induced pancreatitis is multifactorial and involves both direct toxic effects of alcohol and its metabolites on pancreatic cells, as well as indirect mechanisms mediated through the activation of inflammatory and fibrotic pathways (Rasineni *et al*., 2020).

2.2.1.4 Gallbladder

Diseases affecting the gallbladder encompass a wide spectrum, ranging from asymptomatic gallstones to acute and chronic cholecystitis. While the etiology of gallbladder disease is multifactorial, emerging evidence suggests a significant association between alcohol consumption and the development of these conditions, although the underlying mechanisms remain incompletely understood (Cha *et al*., 2017). Epidemiological studies have consistently demonstrated a positive correlation between alcohol intake and the prevalence of gallstones. Indeed, alcohol can affect bile composition and secretion, leading to alterations in cholesterol and bile salt metabolism, which are key factors in the formation of gallstones (Unalp-Arida and Ruhl, 2023). Moreover, heavy alcohol intake is often accompanied by poor dietary habits and obesity, both of which are known risk factors for gallstone development (Shabanzadeh and Novovic, 2017). Moreover, chronic and excessive alcohol consumption is a known risk factor for gallbladder inflammation, which can lead to the development of both acute and chronic cholecystitis (Unalp-Arida and Ruhl, 2023). Alcohol can exacerbate inflammation within the gallbladder by promoting the secretion of inflammatory cytokines and increasing oxidative stress. Furthermore, alcohol abuse can lead to the formation of gallstones, which can obstruct the cystic duct and contribute to the development of cholecystitis.

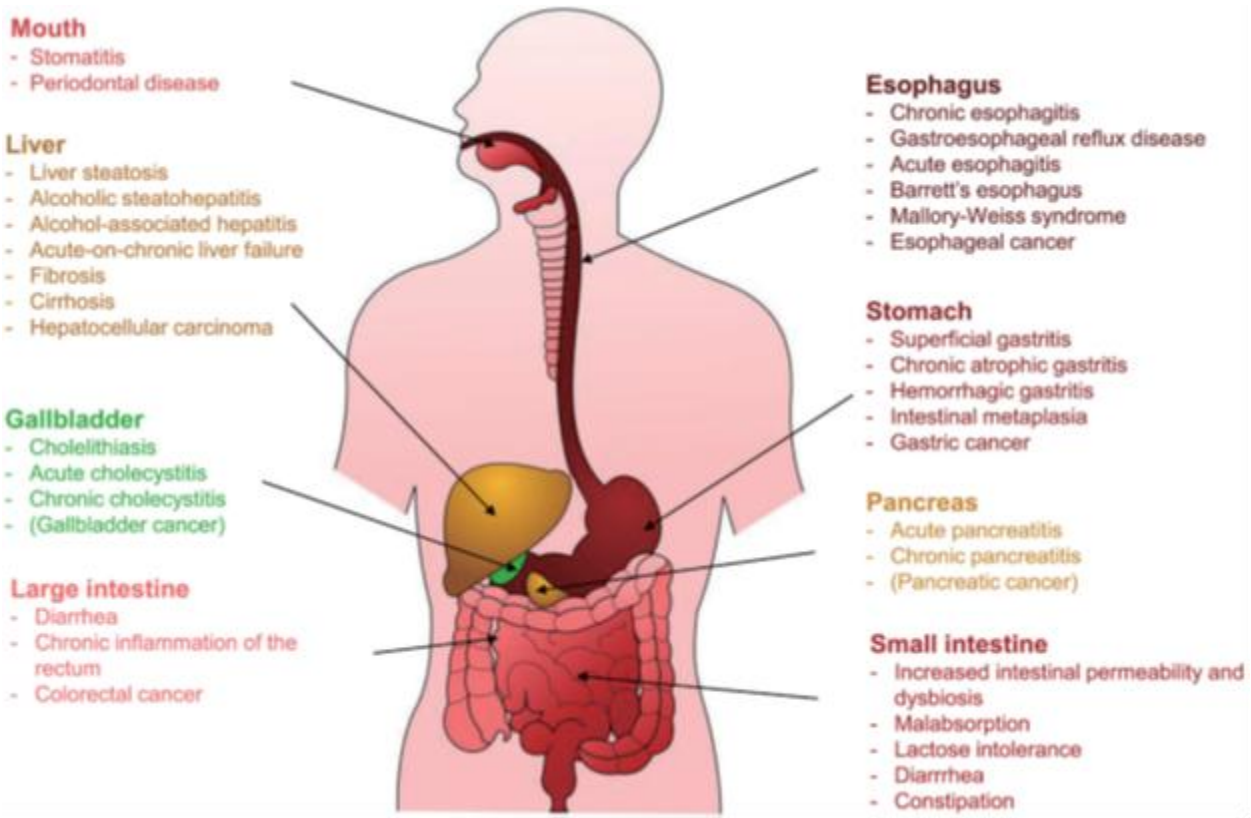


Fig. 1. Acute and chronic pathological effects of ethanol on the digestive system. Brackets indicate an increased risk of developing cancer, although the relationship is not yet fully established.

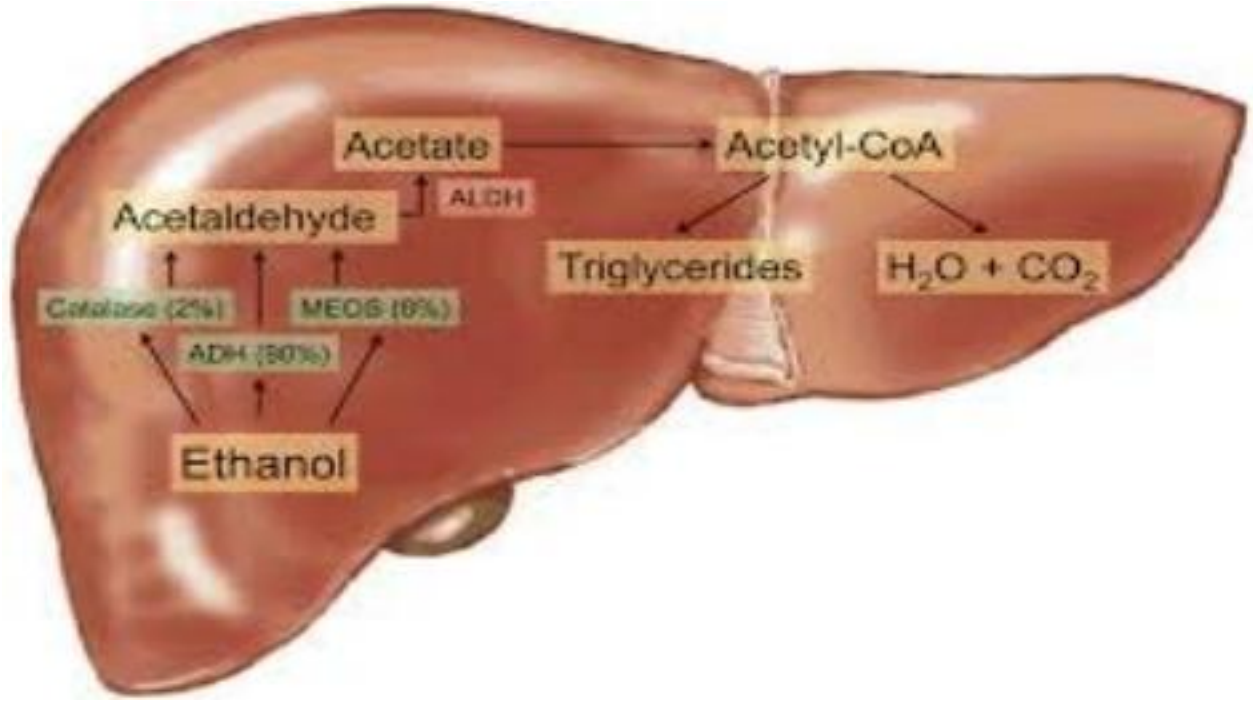


Fig. 2. Diagrams illustrating the various pathways of alcohol metabolism in the liver.

The first step of alcohol metabolism occurs in the liver via the alcohol dehydrogenase enzyme. The subsequent phases involve other key enzymes that form intermediate and final metabolites, such as acetate and acetyl-coenzyme A (acetyl-CoA). ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; MEOS, microsomal ethanol oxidation system.

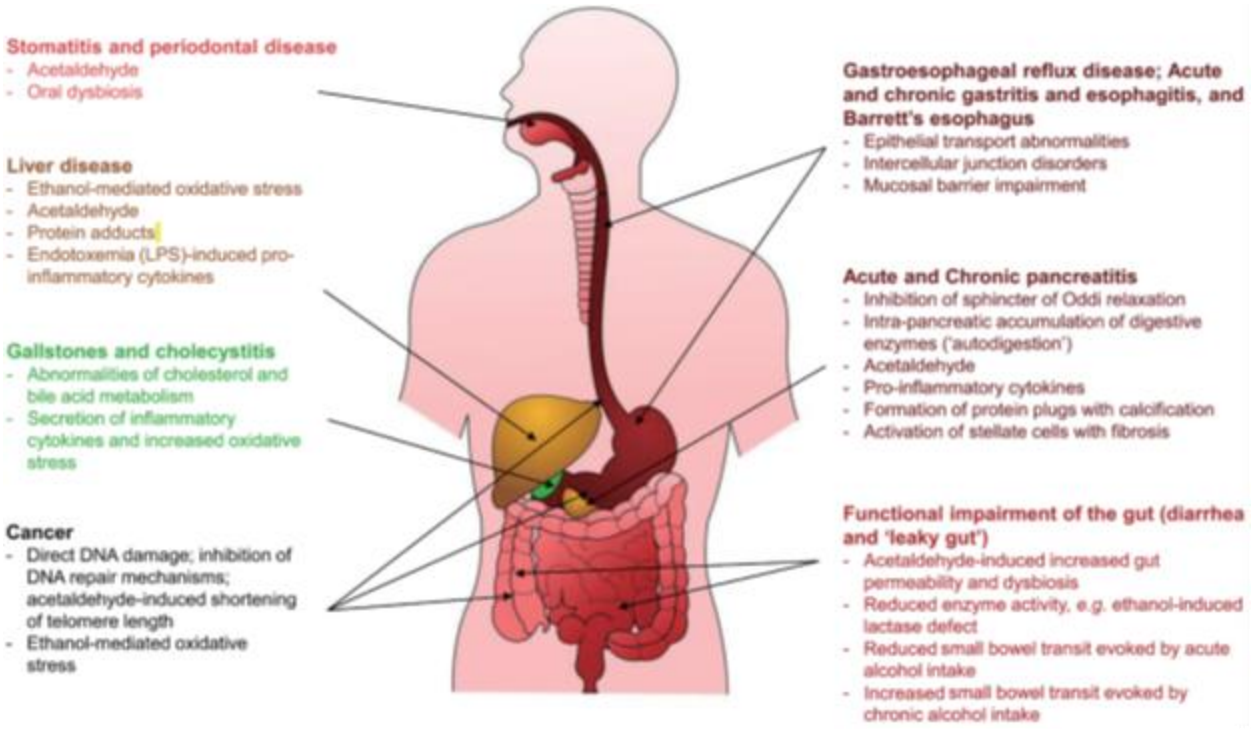


Fig. 3. Main molecular mechanisms and related clinical conditions of alcohol-induced digestive system damage. LPS, lipopolysaccharide.

2.3 Stomach

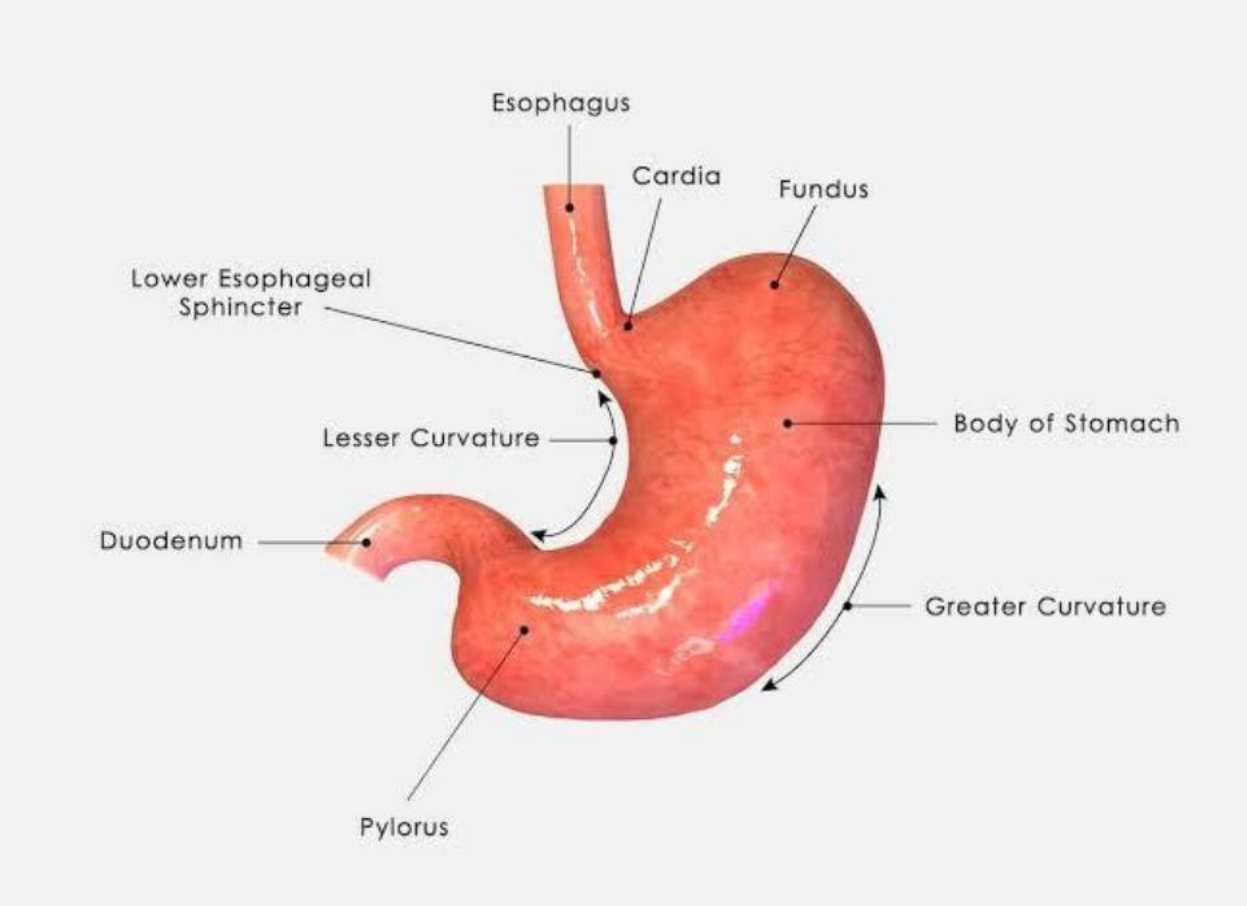
The stomach is an extraordinary organ with vital digestive, nutritional and endocrine functions. The primary function of the stomach is to store food, facilitate digestion, regulate appetite, and aid in absorption of nutrients.

2.3.1 Anatomy of the Stomach

2.3.1.1 Divisions

The stomach is a pear-shaped, asymmetrical, most proximally located organ in the digestive tract (Zuiderma, 1995). It originates as the tubular embryonic foregut dilation during the fifth week of

gestation. By the seventh week, it rotates, dilates, and descends with elongation of the greater curvature into its normal anatomic shape and position (Zuiderma, 1995). After birth, this becomes the most proximal organ of the alimentary tract. The stomach resides in the left upper quadrant of the abdomen. The majority of the amount of the anterior portion of the stomach is covered by the left lateral segment of the liver. Inferiorly, the stomach is attached to the spleen, transverse colon, and caudate lobe of the liver. The hepatogastric ligament, sometimes referred to as pars flacida or the lesser omentum, is bound to the lesser curvature. The gastrosplenic ligament attaches to the spleen to the proximal greater curvature. The cardia is the most proximal region of the stomach and is attached to the esophagus, and the lower esophageal sphincter (LES) is located just proximal to the cardia. The superior most portion of the stomach is fundus that is floppy and distensible, bounded laterally by the spleen and superiorly by the diaphragm. The angle of his is the intersection of the left side of the gastroesophageal (GE) junction. The inferior part of the fundus lies in the horizontal plane of the GE junction, and serves as the transition point to the body (corpus) of the stomach. Although the parietal (oxyntic) cells are mostly in the body of the stomach, they are also present in the cardia and fundus. The body of the stomach is bounded on the right by the lesser curvature which is relatively straight, and the more elongated portion of the greater curvature on the left. The body of the stomach ends where the lesser curvature abruptly angles to the right at the angluris incisura, marking the anatomic beginning of the antrum. The antrum comprises nearly 30% of the distal stomach. Inferiorly, the antrum is connected to the proximal duodenum through the pylorus. The stomach is fixed at the GE junction and pylorus, but is quite mobile at its large midportion (Mulholland, 2006).



2.3.1.2 Arterial and Venous Blood Supply

The stomach is a highly vascularized organ that gets most of its blood supply from the celiac axis (Mulholland, 2006). Four main arteries supply the stomach: the left and right gastric arteries supply the lesser curvature of the stomach, while the left and right gastroepiploic arteries form an arcade around the greater curvature of the stomach. The left gastric artery is the largest artery that supplies the stomach and arises directly off the celiac trunk. In 15-20% of patients, an aberrant left hepatic artery originates from the left gastric artery (Mulholland, 2006). Rarely, is the left hepatic artery the only source of arterial supply to the liver, but inadvertent ligation may result in left sided acute hepatic ischemia. The right gastric artery arises from the hepatic artery and courses along the most inferior portion of the stomach. Behind the first portion of the

duodenum, the right gastroepiploic artery arises from the gastroduodenal artery. The right gastroepiploic artery is the main supply to the gastric conduit after esophagectomy. The left gastroepiploic artery originates from the splenic artery, and forms an anastomotic arcade with the right gastroepiploic artery to supply the greater curvature. The fundus receives blood from the short gastric arteries that arise as branches from the splenic artery. The venous drainage of the stomach usually parallels the arteries. The left gastric (coronary vein) and right gastric veins drain into the portal vein. Occasionally, the coronary vein drains into the splenic vein (Mulholland, 2006). The left gastroepiploic vein drains into the splenic vein, while the right gastroepiploic vein drains into the superior mesenteric vein.

2.3.1.3 Lymphatic Drainage

Generally, the lymphatics parallel the blood vessels. Four zones of lymph nodes have been identified that provide drainage (Bonenkamp *et al*., 1999). The superior gastric group provides drainage for the antrum along the lesser curvature into the right suprapancreatic nodes. The pancreaticolienal nodes drain lymph high on the greater curvature into the left gastroepiploic and splenic nodes. The inferior gastric and subpyloric group of nodes drains lymph along the right gastroepiploic vessels Bonenkamp. Due to its rich lymphatic plexus, the gastric wall can have microscopic evidence of malignant cells several centimeters away from the primary tumor. This is the reason that surgeons perform an extensive lymphadenectomy for gastric cancer.

2.3.1.4 Innervation

The stomach has an extensive extrinsic and intrinsic nerve supply. As represented in the figure below, the vagus nerve provides extrinsic parasympathetic innervation to the stomach, while the celiac plexus provides sympathetic innervation. The vagus nerve originates in the vagal nucleus,

which is located in the floor of the fourth ventricle and travels via the carotid sheath to the mediastinum (Mulholland, 2006). In the mediastinum, the recurrent laryngeal nerve originates from the vagus and divides into several branches around the esophagus. At the esophageal hiatus, these branches combine to produce the right and left vagus nerves. The left vagus nerve runs anteriorly and the right vagus nerve runs posteriorly at the GE junction; this can be remembered using the mnemonic LARP (Mulholland, 2006). The anterior branch gives off a branch to the liver in the gastrohepatic ligament and continues along the lesser curvature as the anterior nerve of Latarjet. The nerves of Latarjet transmit segmental branches to the body of the stomach and terminate in the angularis incisura, forming the crows feet. The criminal nerve of Grassi, the first branch off the posterior vagus, innervates the posterior fundus. If the nerve of Grassi is not divided, it may act as a causative factor for recurring ulcers (Mulholland, 2006). By dividing the crows feet to the proximal stomach and retaining innervation to the antrum and pylorus, a highly selective vagotomy can be accomplished. Truncal vagotomies are performed by ligating the vagus above the celiac and hepatic branches. Sympathetic nerve fibers originate at spinal levels T5–T10 and go to the celiac ganglion via the splanchnic nerves. Postganglionic fibers then move along the arterial system to innervate the stomach. Two nerve plexuses, Auerbach's and Meissner's provide enteric or intrinsic innervation of the stomach. This region consists of a dense collection of serotoninergic, cholinergic, and peptidergic neurons.

2.3.1.5 Histology

The gastric wall is made up of four distinct layers: serosa, muscularis propria, submucosa and mucosa (Owen, 1986). The mucosa, which is the innermost layer, is lined with columnar glandular epithelial cells; the mucosa is composed of three layers: the epithelium, the lamina propria, and the muscularis mucosa. The lamina propria is a layer of connective tissue, blood

vessels, nerve fibers, and inflammatory cells that lies beneath the basement membrane. Depending on their location, gastric glands are lined with different epithelial cells. Endocrine cells such as somatostatin (D) cells and gastrin (G) cells are also present in gastric glands. Mucous secreting surface epithelial cells (SECs) are present throughout the stomach, which secrete bicarbonate and form a protective barrier against injury from pepsin, acid, and ingested irritants. Except for endocrine cells, all epithelial cells in the stomach have the ability to produce bicarbonate (Owen, 1986). The glands of the stomach vary in morphology, according to location. For example, the glands in the cardia tend to be more branched with shorter pits and secrete higher concentrations of bicarbonate than acid. The glands in the fundus and body are more tubular with deeper pits. In the body of the stomach, glands are lined with mostly parietal and chief cells. In the intracellular space, parietal cells release acid, intrinsic factor, and bicarbonate. Pepsinogen is secreted by chief cells (also known as zymogenic cells) and activated at a pH of 2.5. Gastric glands in the antrum are more branching and shallow, containing G cells that secrete gastrin and D cells that secrete somatostatin. Parietal cells, however, are rare in the antrum. The antrum, in general, produces more gastrin than acid, whereas the proximal stomach produces acid rather than gastrin. Parietal cells make up 13% of epithelial cells in the stomach, according to histologic studies. Chief cells account for 44%, mucous cells for 40%, and endocrine cells about 3% (Owen, 1986). Blood arteries, lymphatics, nerve fibers, inflammatory cells, and Meissner's submucosal plexus are all abundant in the submucosa of the stomach. The submucosa and the mucosa give the stomach its characteristic grossly appearing rugal folds (Owen, 1986). Beneath the submucosa lies the muscularis propria also called the muscularis externa. It is composed of two muscular layers, an inner oblique layer, a complete middle circular layer and a complete outer longitudinal layer. This layer contains a rich collection of nerves and autonomic

ganglia, which also comprises Auerbach's myenteric plexus. This layer also contains the interstitial cells of Cajal (ICC) (Owen, 1986). The outermost layer, the serosa is also referred to as the visceral peritoneum. This layer provides significant tensile strength, particularly for anastomoses (Owen, 1986). Microscopic or gross peritoneal metastases are common when tumors that originate in the mucosa penetrate and breach the serosa, presumably due to shedding of tumor cells that would not have occurred if the serosa had not been penetrated. In this way, the serosa might be thought of as the stomach's outer envelope. Microscopic and gross metastases from primary tumors into the stomach are common as a result of penetrating the mucosa and serosa. This would not have occurred in the absence of a penetration of the serosa. In this regard, the serosa can be viewed as an exterior layer that acts as a barrier against tumor infiltration (Owen, 1986).

2.3.2 Physiology of the Stomach

The stomach's primary function is twofold: it acts as a storage vessel for food and it assists the small intestine in digestion and nutrient absorption. The stomach's function is influenced by a variety of neurological and hormonal mediators. Acid production is specific to the stomach and is a central component of the stomach's function during the digestion process. A phenomenon known as receptive relaxation allows the stomach to function as a storage compartment. The proximal region of the stomach relaxes in anticipation of food during normal oral intake (Mercer *et al*., 2002). As a result, liquids move from the stomach and into the small intestine with ease. Solid components settle along the greater curvature of the fundus until they are tiny enough to be released into the small intestine by coordinated antrum propulsions. While the food bolus is being stored in the stomach, it is bathed in acid, which aids in its digestion. Ulceration is

prevented by an intact defense mechanism against mucosal damage caused by the stomach's acidic fluids (Mercer *et al*., 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials: Distilled water, syringes, weighing scale, chloroform, surgical gloves, cotton wool, beakers, plain bottles, universal bottles, laboratory cages, sawdust, formalin, microscope slides, paraffin wax, H. pylori detection kit or culture medium.

3.2 Chemicals: All reagents and chemicals to be used in this study will be of analytical grade.

3.3METHOD

3.3.1 EXPERIMENTAL ANIMAL.

A total of 40 adult Wistar rats weighing 180–200 g will be used. Animals will be housed in standard cages in the animal house in the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Edo State. And acclimatized for two weeks with free access to food and water. Each animal procedure was carried out in accordance with the approved protocols and in compliance with the recommendation for the proper management and utilization of laboratory animals used for research (Liguori*., et al*. 2017).

3.3.2 EXPERIMENTAL DESIGN

Rats will be randomly divided into four groups (n = 10 per group):

Group A (Control)	No treatment.
Group B (Alcohol only)	Administered 1ml of 50% alcohol orally daily
Group C (Melatonin + Alcohol)	Administered 5mg/kg melatonin and 1ml of 50% alcohol orally daily
Group D (Melatonin only	**Administered 5mg/kg of melatonin orally daily**

Treatments will be given daily for 28 days using an oral gavage. The rats were administered with Melatonin and Alcohol simultaneously for 28 days

3.4 METHOD OF SACRIFICE AND TISSUE COLLECTION

At the end of 28 days treatment, the rats were weighed using a weighing scale. Then animals were anesthetized with chloroform for about two minutes and sacrificed. In sacrificing, a midline incision was made through the ventral abdominal wall of each rat. The stomach of each rat was harvested immediately, and put in formalin. The rats were weighed . The stomach tissues were then fixed in 10% formal Saline for routine hematoxylin and eosin histological processing.

3.5 Statistical Analysis

Data will be analyzed using GraphPad Prism Version 8.1. then Results will be expressed as Mean ± Standard Error of Mean (SEM), with significance determined using one-way ANOVA. A p-value of less than 0.05 will be considered statistically significant.

CHAPTER FOUR

RESULTS

STATISTICAL RESULTS

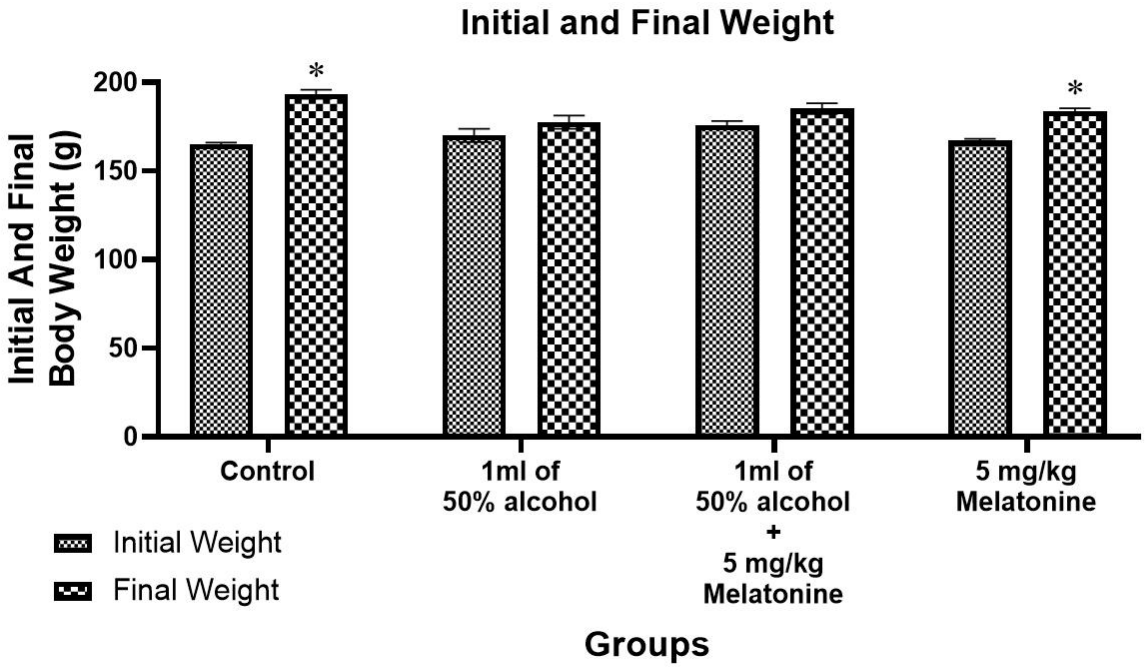


Fig. 1: Initial and Final weight after 28 days of administration Values are given as mean ± SEM. *p< 0.05 compared with the initial weight within group.

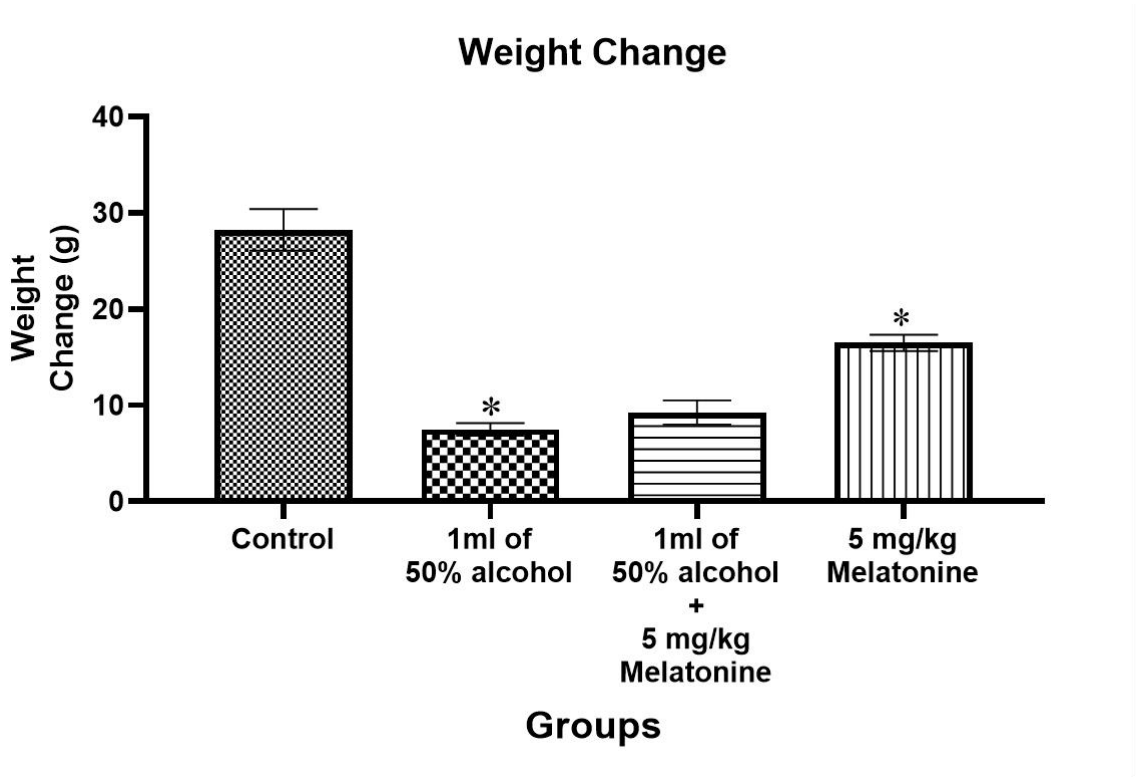


Fig. 2: weight change after 28 days. Values are given as mean ± SEM. *p< 0.05 compared with the control group; $^{\#}p$< 0.05 compared with the 1ml of 50% alcohol alone group.

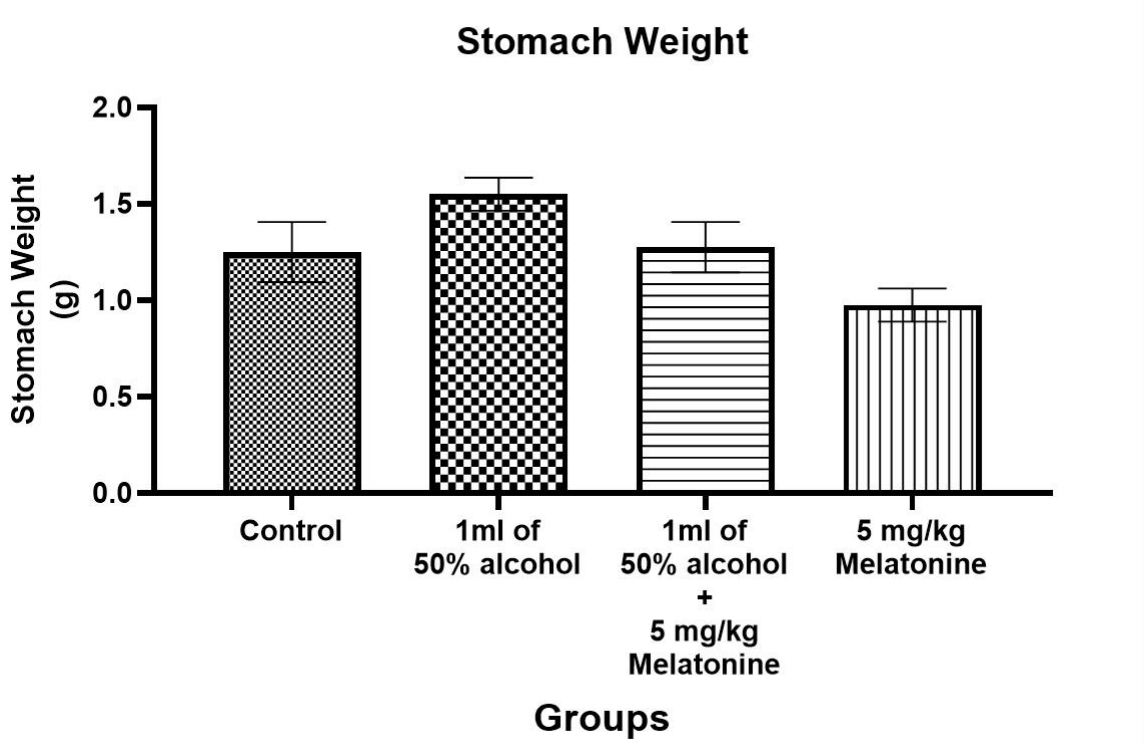


Fig. 3: Stomach weight after 28 days. Values are given as mean ± SEM.

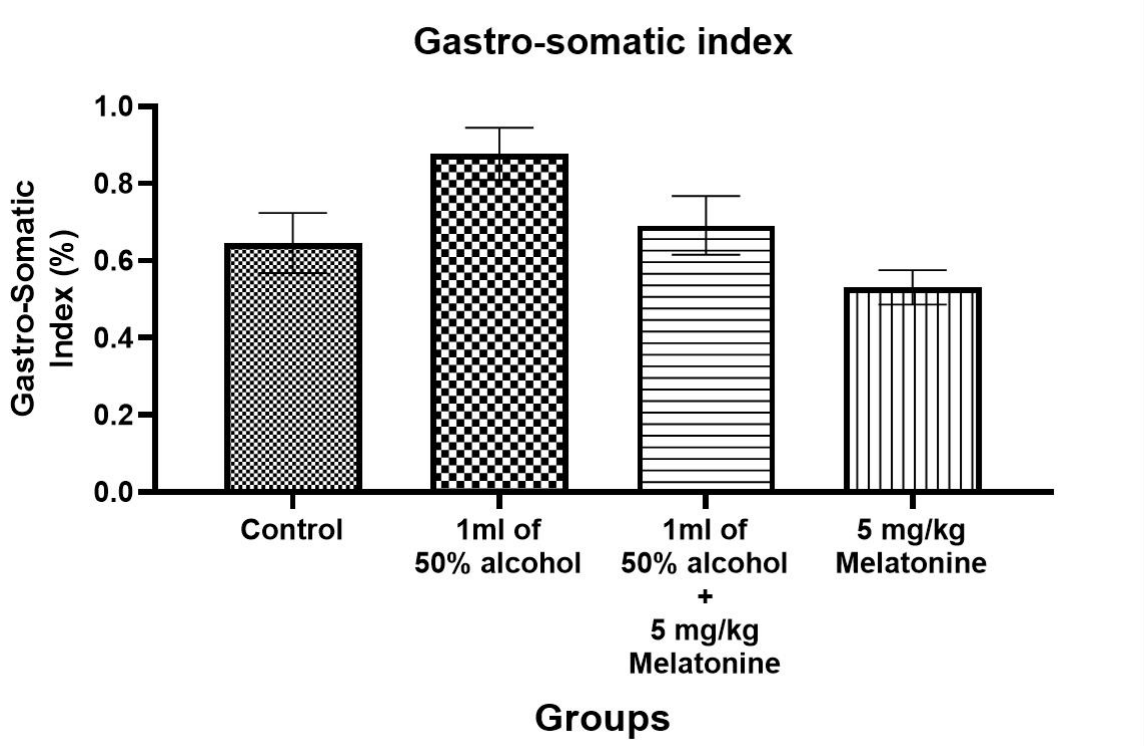


Fig. 4: Gastro-somatic index after 28 days. Values are given as mean ± SEM.

HISTOLOGY

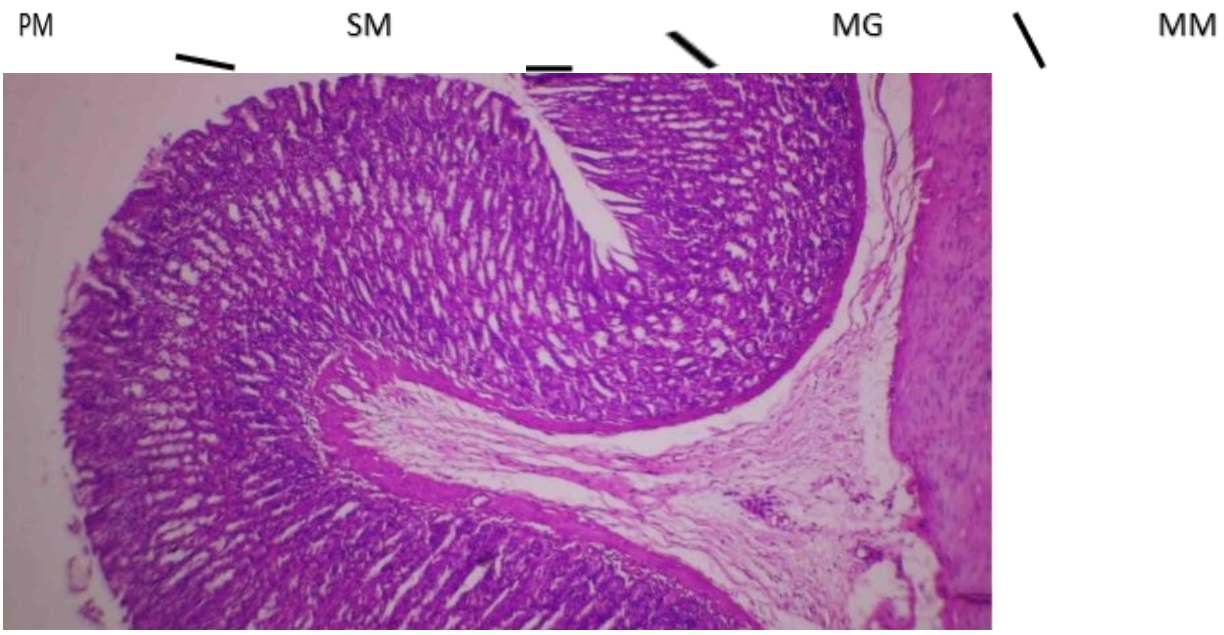


Plate 1. Rat stomach, control, composed of normal architecture: pitting mucosal membrane (PM), mucosal glands (MG), muscularis mucosa (MM) and submucosa (SM): H&E 40 X

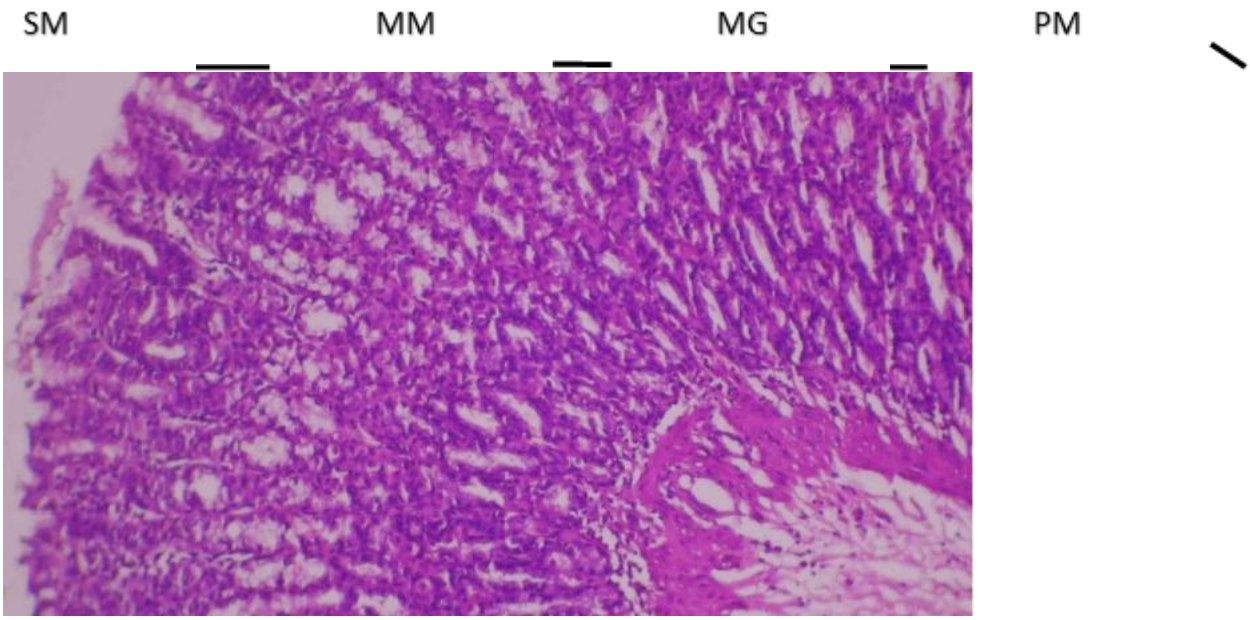


Plate 2. Rat stomach, control, composed of normal architecture: pitting mucosal membrane (PM), mucosal glands (MG), muscularis mucosa (MM) and submucosa (SM) : H&E 100 X

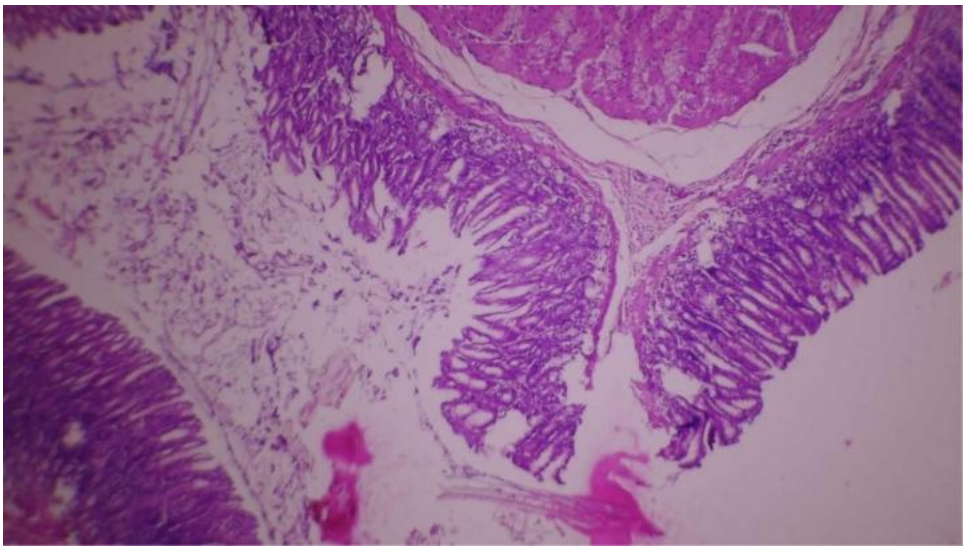


Plate 3. Rat stomach given Alcohol only show: irregularly-shape ulcer UL devitalized mucosal tissue (DM) and muscularis mucosa (MM): H&E 40 X

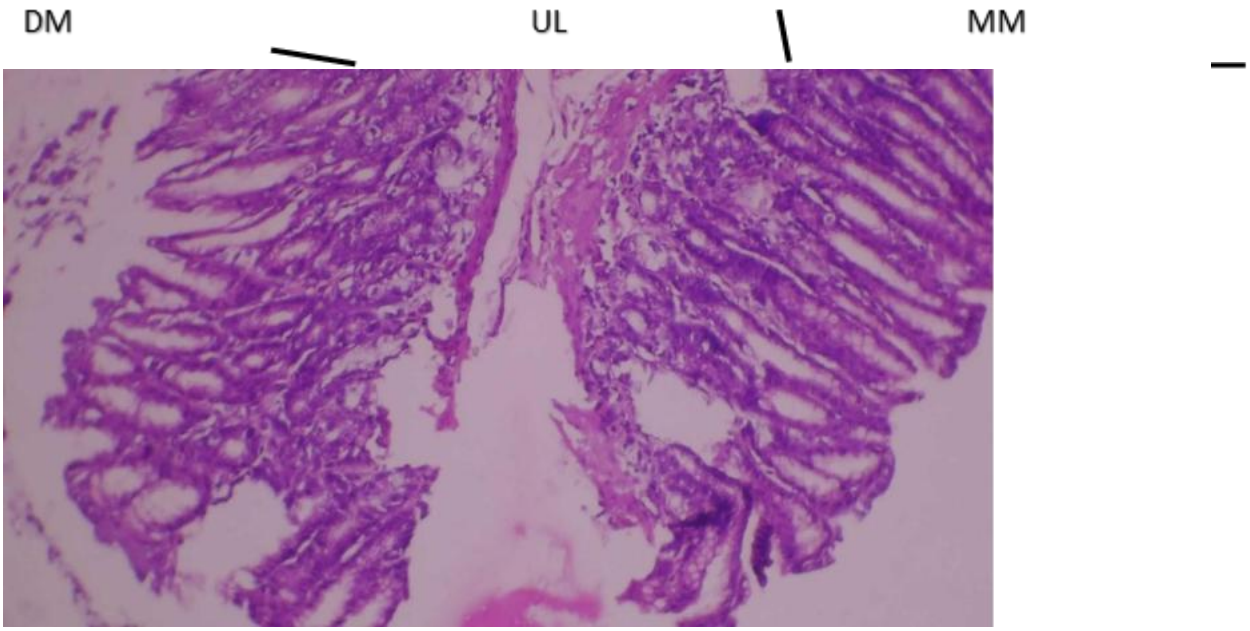


Plate 4. Rat stomach given Alcohol only show: irregularly-shape ulcer (UL), devitalized mucosal tissue (DM) and muscularis mucosa (MM) : H&E 100 X

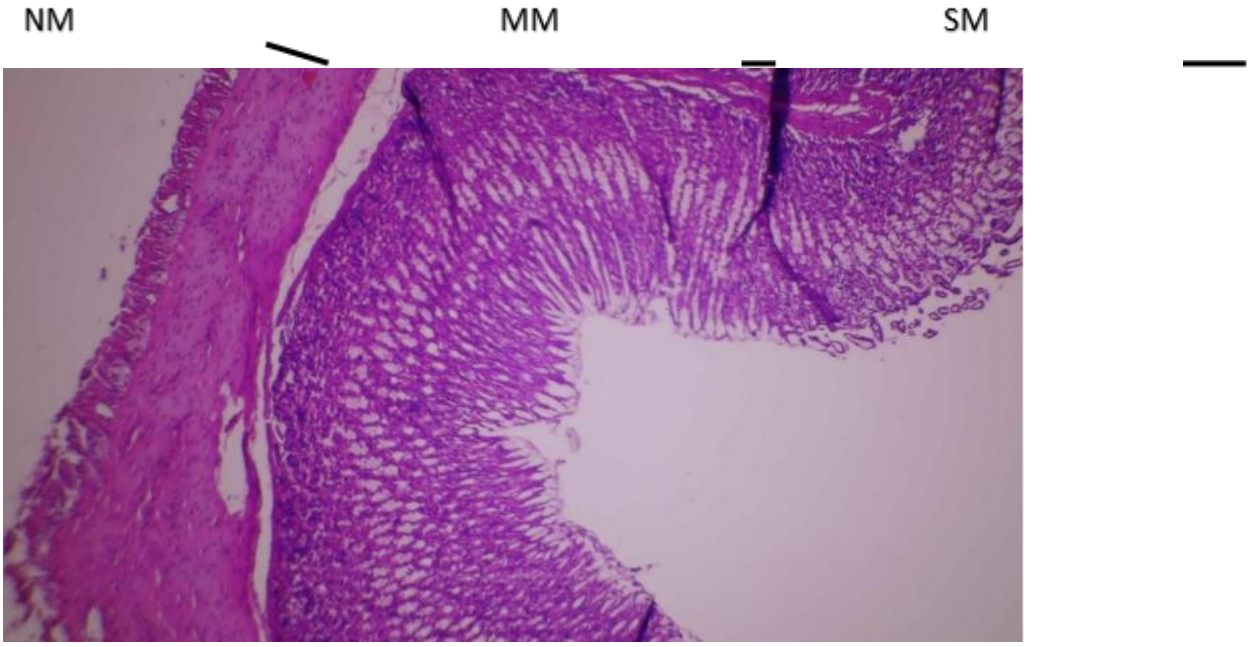


Plate 5. Rat stomach given Melatonin only show: normal mucsle muscularis mucosa (MM) and submucosa (SM): H&E 40 X Plate 6. Rat stomach given Melatonin only show: normal mucosa (NM) muscularis mucosa (MM) and submucosa (SM) : H&E 100 X

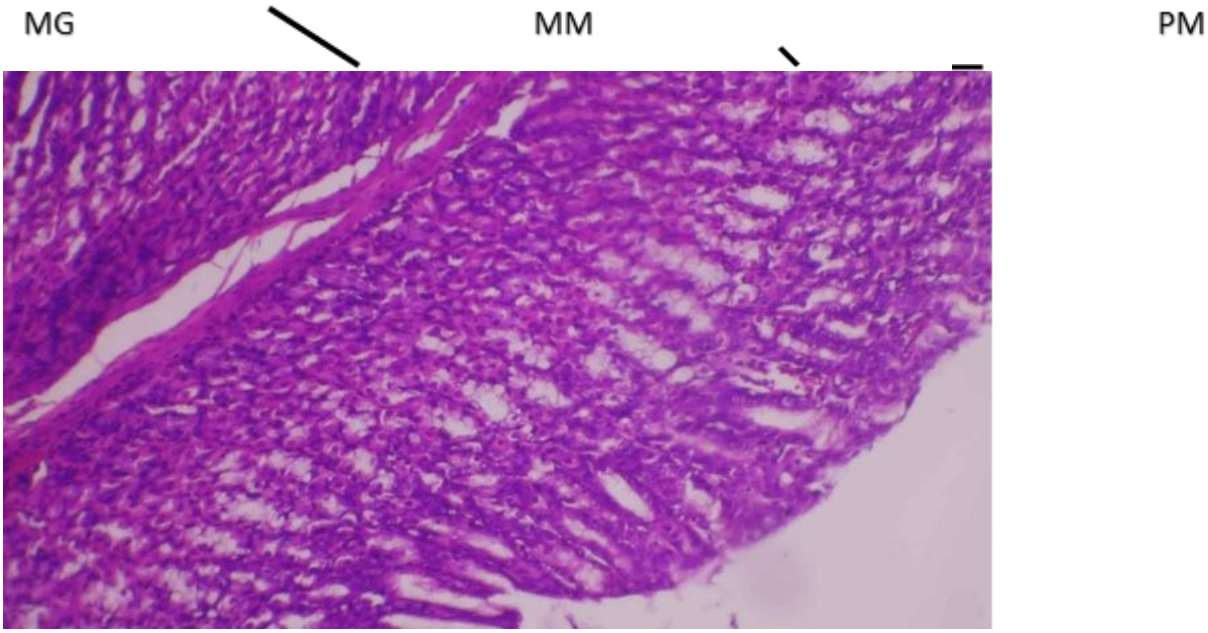


Plate 8. Rat stomach given Alcohol + Melatonin show: normal architecture: pitting mucosal membrane (PM), mucosal glands (MG) and muscularis mucosa (MM) : H&E 100 X

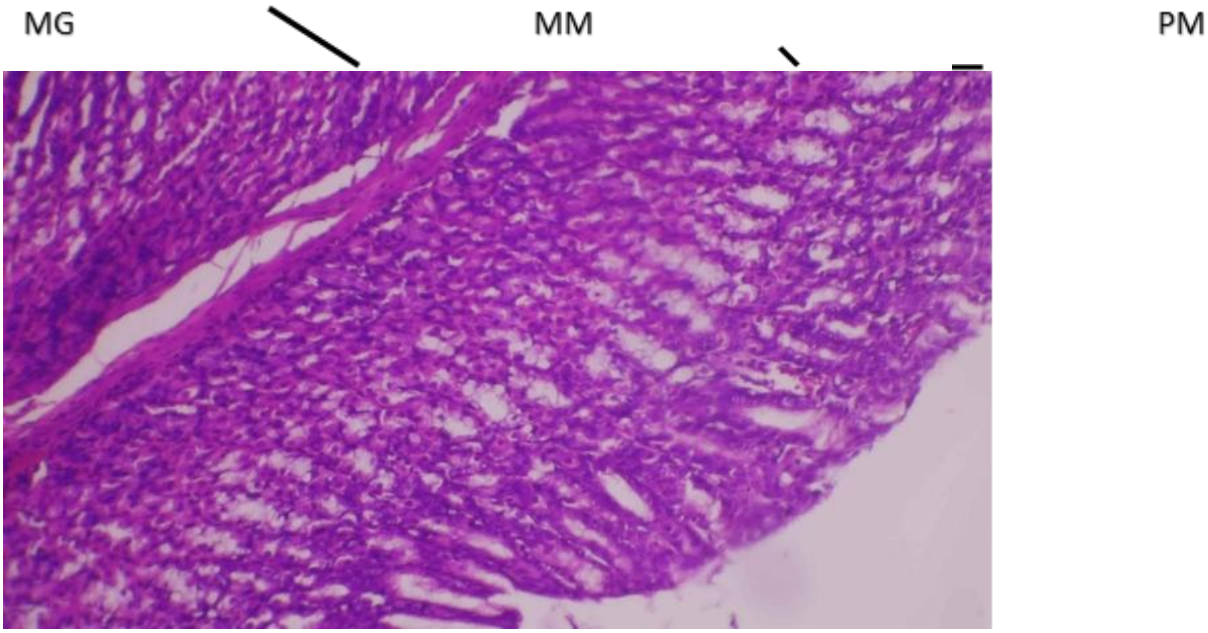


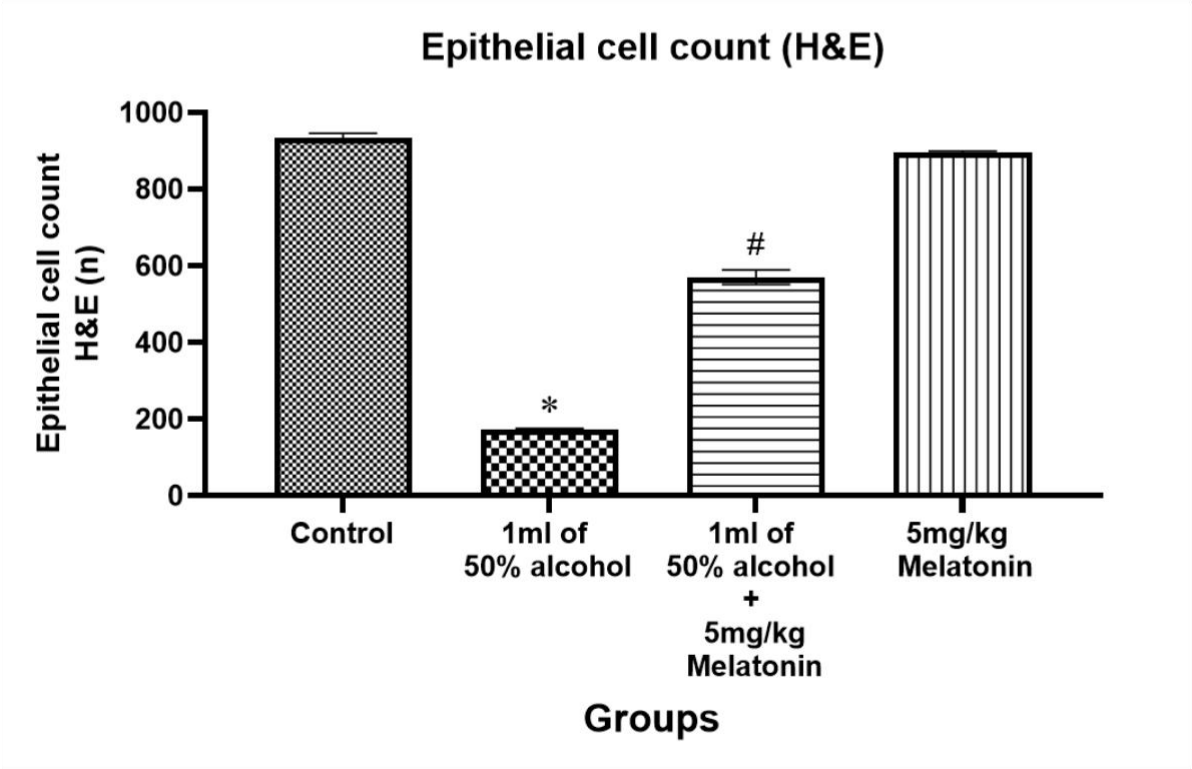
Plate 8. Rat stomach given Alcohol + Melatonin show: normal architecture: pitting mucosal membrane (PM), mucosal glands (MG) and muscularis mucosa (MM) : H&E 100 X

Table 1: Epithelial cell count in the stomach mucosa of Wistar rats following alcohol and melatonin treatment

Groups/Test	Control	1ml of 50% alcohol	1ml of 50% alcohol + 5mg/kg of Melatonin	5mg/kg 0f melatonin	p-value
Epithelial cell count (n)	934.00±12.00	172.50±3.50*	571.00±19.00#	896.50±3.50	<0.001

Values are expressed as mean ± SEM. *p < 0.05 vs. Control; #p < 0.05 vs. Alcohol group.

Chart 1: Graphical representation of epithelial cell count in the stomach mucosa of Wistar rats exposed to 50% alcohol and/or melatonin treatment.



Values are expressed as mean ± SEM. *p < 0.05 vs. Control; #p < 0.05 vs. Alcohol group.

CHAPTER FIVE

DISCUSSION AND CONCLUSION.

5.1 DISSCSION

Alcohol is a well-known toxin and a major risk factor for several diseases, particularly affecting the gastrointestinal tract and the brain. Chronic or excessive alcohol consumption is a potent ulcerogenic agent. It causes severe damage to the gastric mucosal tissue, leading to the formation of irregularly shaped ulcers that can penetrate deeply into the stomach wall, often extending to the muscularis mucosa.

The principal aim of this study was to investigate the effects of melatonin on alcohol-induced gastric toxicity in adult Wistar rats. The histopathological and morphometric findings provide compelling evidence that chronic alcohol administration induces severe gastric mucosal injury, and that melatonin co-administration effectively mitigates this damage. The gastric tissue from the control group and the melatonin-only group both exhibited normal tissue architecture, characterized by a well-defined mucosal membrane, intact gastric pits, mucosal glands (MG), muscularis mucosa (MM), and submucosa (SM) . This integrity provides the essential background for comparison, reaffirming that melatonin alone does not adversely affect the normal stomach structure.

In stark contrast, the rats administered alcohol only displayed significant pathological lesions, including an irregularly-shaped ulcer (UL) and remarkable areas of devitalized mucosal tissue (DM) . The severity of the alcohol-induced injury was evident as the ulcer extended deeply into the muscularis mucosa (MM). This finding aligns with established literature Sun, A. Y., Ingelman-Sundberg *et al*., (2001) that documents alcohol's toxic effects on the gastrointestinal

tract, where it compromises the mucosal barrier and leads to irritation and inflammation, increasing the risk of peptic ulcer disease. The mechanism is often attributed to the generation of reactive oxygen species (ROS) and acetaldehyde as metabolic by-products, which cause oxidative damage and compromise the mucosal barrier

Crucially, the Alcohol + Melatonin group showed a striking resolution of the ulcer, with the gastric tissue presenting a normal architecture similar to that of the control group. This included a healthy pitting mucosal membrane (PM), mucosal glands (MG), and muscularis mucosa (MM) . This protective effect strongly supports the initial hypothesis that melatonin is gastroprotective.

The morphometric analysis further substantiated the histopathological observations, revealing that the alcohol-only group had the lowest count of viable cells in the gastric mucosa. Conversely, the melatonin-only group had the highest viable cell count, compared to the control group. The Alcohol + Melatonin group demonstrated a cell count value that was a close distance behind the control group. This suggests that alcohol destroyed a considerable mass of gastric mucosal cells, a degree of devitalization which was effectively resolved by melatonin. Melatonin's protective action is likely multifaceted. Melatonin, an endogenous hormone synthesized in the pineal gland and the gastrointestinal tract itself, possesses anti-inflammatory, anti-secretory, and mucosal barrier-stabilizing properties (Bubenik, 2002). Its role as a powerful antioxidant is well-documented. In the context of this study, the observed increase in gastric mucosa tissue mass in the melatonin-treated groups suggests that melatonin likely resolved the ulcer by stimulating the regeneration of dead cells, effectively countering the oxidative stress and damage induced by alcohol.

5.2. CONCLUSION

Based on the histopathological and morphometric findings of this study, it is concluded that alcohol consumption induces severe gastric ulceration in adult Wistar rats. Furthermore, the administration of melatonin demonstrates a potent gastroprotective effect by preventing and resolving alcohol-induced gastric toxcicity. This protective mechanism is strongly inferred to involve the stimulation of dead cell regeneration and recovery of the gastric mucosal tissue mass, highlighting melatonin's capacity to restore normal tissue architecture after acute toxic injury.

References

Ali, A., & AlHussaini, K. I. (2024). Helicobacter pylori: a contemporary perspective on pathogenesis, diagnosis and treatment strategies. *Microorganisms*, *12*(1), 222.

Blask D.E, Dauchy R.T, Sauer L.A. (2005). Putting cancer to sleep at night: the neuroendocrine/circadian melatonin signal. *Endocrine*, **27**:179–188.

Bonenkamp J.J, Hermans J, Sasako M, van de Velde C.J. (1999) Extended lymph-node dissection for gastric cancer. Dutch Gastric Cancer Group. *N Engl J Med*, **340**: 908-914.

Brzoska M.M, Moniuszko-Jakonink J, Pilat-Marcinkieewicz B, Sawicki B. (2003). Liver and kidney function and histology in rats exposed to cadmium and ethanol. *Alcohol Alcohol*; **38**:2–10.

Bubenik G.A. (2002). Gastrointestinal melatonin: localization, function, and clinical relevance. *Dig. Dis. Sci*, **47**:2336–2348.

Bujanda L. (2000). The effects of alcohol consumption upon the gastrointestinal tract. *Am J Gastroenterol*, **95**(12):3374–3382.

Carrillo-Vico A, Calvo J.R, Abreu P, Lardone P.J, Garcia-Maurino S, Reiter R.J, Guerrero J.M. (2004). Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance. *FASEB J*, **18**:537–539.

Cha B.H, Lee B.S, Lee S.H, Kang S.J, Park M.J. (2017). A Study of Alcohol Consumption and Obesity as Main Risk Factor for Symptomatic Gallbladder Stone: a Case-Control Study. *Asian Pac J Cancer Prev*, **18**(3):715– 719.

Chen T.Y, Lee M.Y, Chen H.Y, Kuo Y.L, Lin S.C, Wu T.S, Lee E.J. (2006). Melatonin attenuates the postischemic increase in blood-brain barrier permeability and decreases hemorrhagic transformation of tissue-plasminogen activator therapy following ischemic stroke in mice. *J. Pineal Res*, **40**:242–250.

Claustrat B, Brun J, Chazot G. (2005). The basic physiology and pathophysiology of melatonin. *Sleep Med. Rev*, **9**(1):11–24.

Coon S.L, Roseboom P.H, Baler R, Weller J.L, Namboodiri M.A, Koonin E.V, Klein D.C. (1995). Pineal serotonin N-acetyltrans- ferase: expression cloning and molecular analysis. *Science*, **270**(5242):1681–1683.

Di W.L, Kadva A, Johnston A, Silman R. (1997). Variable bioavailability of oral melatonin. *N. Engl. J. Med*, **336**(14):1028–1029.

Dubocovich M.L. (1995). Melatonin receptors: are there multiple subtypes? *Trends Pharmacol. Sci*, **16**(2):50–56.

Ebisawa T, Karne S, Lerner M.R, Reppert S.M. (1994). Expression cloning of a high affinity melatonin receptor from Xenopus dermal melanophores. *Proc. Natl. Acad. Sci. USA*, **91**(13):6133–6137.

Ekmekcioglu C. (2006). Melatonin receptors in humans: biological role and clinical relevance. *Biomed. Pharmacother*, **60**:97–108.

Ferrua M and Singh R. (2010). *J. Food Sci*; **75**(7):R151.

Haber P.S and Kortt N.C. (2021). Alcohol use disorder and the gut. *Addiction*. **116**(3):658–667.

Hardeland, R. (2017). Melatonin—more than just a pineal hormone. *Biomed. J. Sci. Tech. Res*, *1*(4), 1-4.

Jockers R, Maurice P, Boutin J.A, Delagrange P. (2008). Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? *Br. J. Pharmacol*, **154**(6):1182–1195.

Joseph D, Chong N.W, Shanks M.E, Rosato E, Taub N.A, Petersen S.A. (2014). Getting rhythm: how do babies do it? *Arch. Dis. Child. Fetal Neonatal Ed*, **100**(1): F50–F54.

Karasek K and Winczyk K. (2006). Melatonin in humans. *J. Physiol. Pharmacol*, 57(5):19–39.

Kilic E, Kilic U, Bacigaluppi M, Guo Z, Ben Abdallah N, Wolfer D.P, Reiter R.J, Hermann D.M, Bassetti C.L. (2008). Delayed melatonin administration promotes neuronal survival, neurogenesis and motor recovery, and attenuates hyperactivity and anxiety after mild focal cerebral ischemia in mice. *J. Pineal Res*, **45**:142–148.

Kilic E, Ozdemir Y.G, Bolay H, Kelestimur H, Dalkara T. (1999). Pinealectomy aggravates and melatonin administration attenuates brain damage in focal ischemia. *J. Cereb. Blood Flow Metab*, **19**:511–516.

Klausner E.A, Lavy E, Friedman M, Hoffman J. (2003). Control. Release; 90(2): 143.

Kong X, Li X, Cai Z, Yang N, Liu Y, Shu J, Pan L, Zuo P. (2008). Melatonin regulates the viability and differentiation of rat midbrain neural stem cells. *Cell. Mol. Neurobiol*, **28**(4):569–579.

Konturek, S. J., Konturek, P. C., Brzozowski, T., & Bubenik, G. A. (2007). Role of melatonin in upper gastrointestinal tract. *Journal of physiology and pharmacology*, *58*(6), 23-52.

Lerner A.B, Case J.D, Takahashi Y, Lee T.H, Mori W. (1958). Isolation of melatonin, the pineal gland factor that lightens melanocytes.

Lieber C.S. (2003). Relationships between nutrition, alcohol use, and liver disease. *Alcohol Res Health*; **27**:220–231.

Mahieu S, Contini Mdel C, González M, Millen N. (2009). Melatonin reduces oxidative damage induced by aluminium in rat kidney. *Toxicol Lett*; **190**(1):9–15.

Mercer D.W, Liu T.H, Castaneda A. (2002). Anatomy and physiology of the stomach. In: Yeo CJ, editor. Ahackelford's surgery of the alimentary tract. 5th edtion. Elsevier; p 3.

Morera-Fumero A.L and Abreu-Gonzalez P. (2013). Role of melatonin in schizophrenia. *Int. J. Mol. Sci*, **14**:9037–9050.

Mulholland M.W. (2006). Gastric anatomy and physiology. In: Mulholland MW, editor. Greenfield's surgery: scientific principles and practice. 4th edition. Philadelphia: Lippincott Williams and Wilkins. p 712-720.

Na, H. K., and Lee, J. Y. (2017). Molecular basis of alcohol-related gastric and colon cancer. *International journal of molecular sciences*, *18*(6), 1116.

Nabavi, S. M., Nabavi, S. F., Sureda, A., Xiao, J., Dehpour, A. R., Shirooie, S., ... & Daglia, M. (2019). Anti-inflammatory effects of Melatonin: A mechanistic review. *Critical reviews in food science and nutrition*, *59*(sup1), S4-S16.

Nutt, D., Hayes, A., Fonville, L., Zafar, R., Palmer, E. O., Paterson, L., and Lingford-Hughes, A. (2021). Alcohol and the brain. *Nutrients*, *13*(11), 3938.

Obayuwana E., Imafidon EO., Enoghase JR., and Nweke SM. (2024). The role of aqueous Azadirachta indica leaf extract on alcohol-induced kidney toxicity in adult wistar

rats. *International Journal of Innovation Scientific Research and Review*, *6*(7), 6670-6673.

Obayuwana, E., and Okereke, J. N. (2024). Evaluating the ameliorative effect of aqueous Azadirachta indica leaf extract on alcohol-induced liver damage in adult Wistar rats. *Journal Of Experimental and Clinical Anatomy*, *21*(1), 59-65.

Owen D.A. (1986). Normal Histology of the Stomach. *The Am J of Pathology*, **10**(1): 48-61.

Parekh R.S and Klag M.J. (2001). Alcohol: Role in the development of hypertension and end-stage renal disease. *Curr Opin Nephrol Hypertens*; **10**:385–390.

Pires-Lapa M.A, Tamura E.K, Salustiano E.M, Markus R.P. (2013). Melatonin synthesis in human colostrum mononuclear cells enhances dectin-1-mediated phagocytosis by mononuclear cells.

Qu, B., Su, J., Wang, Z., Wang, Y., Han, X., Wang, H., ... & Ren, G. (2015). Effect of H. pylori infection on cytokine profiles and oxidative balance in subjects with chronic alcohol ingestion. *PLoS One*, *10*(6), e0129352.[i]

Rasineni K, Srinivasan M.P, Balamurugan A.N, Kaphalia B.S, Wang S, Ding W.X. (2020). Recent Advances in Understanding the Complexity of Alcohol-Induced Pancreatic Dysfunction and Pancreatitis Development. *Biomolecules*, **10**(5):669.

Rodrigo R, Trujillio M.D, Bosco C, Orellana M, Thielmann L, Araya J. (2002). Changes in (Na+K)-adenosine triphosphatase activity and ultrastructure of lung and kidney associated with oxidative stress induced by acute ethanol intoxication. *Chest*; **121**:589–596.

Sadeh A. (1997). Sleep and melatonin in infants: a preliminary study. *Sleep*, **20**(3):185–191.

Seitz H.K, Egerer G, Simanowski U.A, Waldherr R, Eckey R, Agarwal D.P, Goedde H.W. (1993). Human gastric alcohol dehydrogenase activity: effect of age, sex, and alcoholism. *Gut*, 34.

Shabanzadeh D.M and Novovic S. (2017). Alcohol, smoking and benign hepato biliary disease. *Best Pract Res Clin Gastroenterol*, **31**(5):519– 527

Simonneaux V and Ribelayga C. (2003). Generation of the melatonin endocrine message in mammals: A review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. *Pharmacol. Rev*, **55**:325–395.

Sun A.Y, Ingelman-Sundberg M, Neve E. (2001). Ethanol and oxidative stress. *Alcohol Clin Exp Res*; **25**(5):237–243.

Unalp-Arida A and Ruhl C.E. (2023). Increasing gallstone disease prevalence and associations with gallbladder and biliary tract mortality in the US. *Hepatology*, **77**(6):1882–1895

World Health Organization. (2018). Global status report on alcohol and health 2018. Geneva: World Health Organization.

Yadav D and Lowenfels A.B. (2013). The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology*, **144**(6):1252–1261.

You M and Crabb D.W. (2004). Recent advances in alcoholic liver disease II. Minireview: Molecular mechanisms of alcoholic fatty liver. *Am J Physiol Gastrointest Liver Physiol*; **6**:1–6.

Zawilska J.B, Skene D.J, Arendt J. (2009). Physiology and pharmacology of melatonin in relation to biological rhythms.

Zuiderma G. (1995). Shackelford's Surgery of the Alimentary Tract, 4th ed. Philadelphia, WB Saunders.

---

i

Ali, A., & AlHussaini, K. I. (2024). Helicobacter pylori: a contemporary perspective on pathogenesis, diagnosis and treatment strategies. *Microorganisms*, *12*(1), 222.

Hardeland, R. (2017). Melatonin—more than just a pineal hormone. *Biomed. J. Sci. Tech. Res*, *1*(4), 1-4.

Konturek, S. J., Konturek, P. C., Brzozowski, T., & Bubenik, G. A. (2007). Role of melatonin in upper gastrointestinal tract. *Journal of physiology and pharmacology*, *58*(6), 23-52.

Na, H. K., and Lee, J. Y. (2017). Molecular basis of alcohol-related gastric and colon cancer. *International journal of molecular sciences*, *18*(6), 1116.

Nabavi, S. M., Nabavi, S. F., Sureda, A., Xiao, J., Dehpour, A. R., Shirooie, S., ... & Daglia, M. (2019). Anti-inflammatory effects of Melatonin: A mechanistic review. *Critical reviews in food science and nutrition*, *59*(sup1), S4-S16.

Nutt, D., Hayes, A., Fonville, L., Zafar, R., Palmer, E. O., Paterson, L., and Lingford-Hughes, A. (2021). Alcohol and the brain. *Nutrients*, *13*(11), 3938.

Obayuwana E., Imafidon EO., Enoghase JR., and Nweke SM. (2024). The role of aqueous Azadirachta indica leaf extract on alcohol-induced kidney toxicity in adult wistar rats. *International Journal of Innovation Scientific Research and Review*, *6*(7), 6670-6673.

Obayuwana, E., and Okereke, J. N. (2024). Evaluating the ameliorative effect of aqueous Azadirachta indica leaf extract on alcohol-induced liver damage in adult Wistar rats. *Journal Of Experimental and Clinical Anatomy*, *21*(1), 59-65.

Qu, B., Su, J., Wang, Z., Wang, Y., Han, X., Wang, H., ... & Ren, G. (2015). Effect of H. pylori infection on cytokine profiles and oxidative balance in subjects with chronic alcohol ingestion. *PLoS One*, *10*(6), e0129352.

Blask D.E, Dauchy R.T, Sauer L.A. (2005). Putting cancer to sleep at night: the neuroendocrine/circadian melatonin signal. *Endocrine*, **27**:179–188.

Bonenkamp J.J, Hermans J, Sasako M, van de Velde C.J. (1999) Extended lymph-node dissection for gastric cancer. Dutch Gastric Cancer Group. *N Engl J Med*, **340**: 908-914.

Brzoska M.M, Moniuszko-Jakonink J, Pilat-Marcinkieewicz B, Sawicki B. (2003). Liver and kidney function and histology in rats exposed to cadmium and ethanol. *Alcohol Alcohol*; **38**:2–10.

Bubenik G.A. (2002). Gastrointestinal melatonin: localization, function, and clinical relevance. *Dig. Dis. Sci*, **47**:2336–2348.

Bujanda L. (2000). The effects of alcohol consumption upon the gastrointestinal tract. *Am J Gastroenterol*, **95**(12):3374–3382.

Carrillo-Vico A, Calvo J.R, Abreu P, Lardone P.J, Garcia-Maurino S, Reiter R.J, Guerrero J.M. (2004). Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance. *FASEB J*, **18**:537–539.

Cha B.H, Lee B.S, Lee S.H, Kang S.J, Park M.J. (2017). A Study of Alcohol Consumption and Obesity as Main Risk Factor for Symptomatic Gallbladder Stone: a Case-Control Study. *Asian Pac J Cancer Prev*, **18**(3):715– 719.

Chen T.Y, Lee M.Y, Chen H.Y, Kuo Y.L, Lin S.C, Wu T.S, Lee E.J. (2006). Melatonin attenuates the postischemic increase in blood-brain barrier permeability and decreases hemorrhagic transformation of tissue-plasminogen activator therapy following ischemic stroke in mice. *J. Pineal Res*, **40**:242–250.

Claustrat B, Brun J, Chazot G. (2005). The basic physiology and pathophysiology of melatonin. *Sleep Med. Rev*, **9**(1):11–24.

Coon S.L, Roseboom P.H, Baler R, Weller J.L, Namboodiri M.A, Koonin E.V, Klein D.C. (1995). Pineal serotonin N-acetyltrans- ferase: expression cloning and molecular analysis. *Science*, **270**(5242):1681–1683.

Di W.L, Kadva A, Johnston A, Silman R. (1997). Variable bioavailability of oral melatonin. *N. Engl. J. Med*, **336**(14):1028–1029.

Dubocovich M.L. (1995). Melatonin receptors: are there multiple subtypes? *Trends Pharmacol. Sci*, **16**(2):50–56.

Ebisawa T, Karne S, Lerner M.R, Reppert S.M. (1994). Expression cloning of a high affinity melatonin receptor from Xenopus dermal melanophores. *Proc. Natl. Acad. Sci. USA*, **91**(13):6133–6137.

Ekmekcioglu C. (2006). Melatonin receptors in humans: biological role and clinical relevance. *Biomed. Pharmacother*, **60**:97–108.

Ferrua M and Singh R. (2010). *J. Food Sci*; **75**(7):R151.

Haber P.S and Kortt N.C. (2021). Alcohol use disorder and the gut. *Addiction*. **116**(3):658–667.

Jockers R, Maurice P, Boutin J.A, Delagrange P. (2008). Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? *Br. J. Pharmacol*, **154**(6):1182–1195.

Joseph D, Chong N.W, Shanks M.E, Rosato E, Taub N.A, Petersen S.A. (2014). Getting rhythm: how do babies do it? *Arch. Dis. Child. Fetal Neonatal Ed*, **100**(1): F50–F54.

Karasek K and Winczyk K. (2006). Melatonin in humans. *J. Physiol. Pharmacol*, 57(5):19–39.

Kilic E, Kilic U, Bacigaluppi M, Guo Z, Ben Abdallah N, Wolfer D.P, Reiter R.J, Hermann D.M, Bassetti C.L. (2008). Delayed melatonin administration promotes neuronal survival, neurogenesis and motor recovery, and attenuates hyperactivity and anxiety after mild focal cerebral ischemia in mice. *J. Pineal Res*, **45**:142–148.

Kilic E, Ozdemir Y.G, Bolay H, Kelestimur H, Dalkara T. (1999). Pinealectomy aggravates and melatonin administration attenuates brain damage in focal ischemia. *J. Cereb. Blood Flow Metab*, **19**:511–516.

Klausner E.A, Lavy E, Friedman M, Hoffman J. (2003). Control. Release; 90(2): 143.

Kong X, Li X, Cai Z, Yang N, Liu Y, Shu J, Pan L, Zuo P. (2008). Melatonin regulates the viability and differentiation of rat midbrain neural stem cells. *Cell. Mol. Neurobiol*, **28**(4):569–579.

Lerner A.B, Case J.D, Takahashi Y, Lee T.H, Mori W. (1958). Isolation of melatonin, the pineal gland factor that lightens melanocytes.

Lieber C.S. (2003). Relationships between nutrition, alcohol use, and liver disease. *Alcohol Res Health*; **27**:220–231.

Mahieu S, Contini Mdel C, González M, Millen N. (2009). Melatonin reduces oxidative damage induced by aluminium in rat kidney. *Toxicol Lett*; **190**(1):9–15.

Mercer D.W, Liu T.H, Castaneda A. (2002). Anatomy and physiology of the stomach. In: Yeo CJ, editor. Ahackelford's surgery of the alimentary tract. 5th edtion. Elsevier; p 3.

Morera-Fumero A.L and Abreu-Gonzalez P. (2013). Role of melatonin in schizophrenia. *Int. J. Mol. Sci*, **14**:9037–9050.

Mulholland M.W. (2006). Gastric anatomy and physiology. In: Mulholland MW, editor. Greenfield's surgery: scientific principles and practice. 4th edition. Philadelphia: Lippincott Williams and Wilkins. p 712-720.

Owen D.A. (1986). Normal Histology of the Stomach. *The Am J of Pathology*, **10**(1): 48-61.

Parekh R.S and Klag M.J. (2001). Alcohol: Role in the development of hypertension and end-stage renal disease. *Curr Opin Nephrol Hypertens*; **10**:385–390.

Pires-Lapa M.A, Tamura E.K, Salustiano E.M, Markus R.P. (2013). Melatonin synthesis in human colostrum mononuclear cells enhances dectin-1-mediated phagocytosis by mononuclear cells.

Rasineni K, Srinivasan M.P, Balamurugan A.N, Kaphalia B.S, Wang S, Ding W.X. (2020). Recent Advances in Understanding the Complexity of Alcohol-Induced Pancreatic Dysfunction and Pancreatitis Development. *Biomolecules*, **10**(5):669.

Rodrigo R, Trujillio M.D, Bosco C, Orellana M, Thielmann L, Araya J. (2002). Changes in (Na+K)-adenosine triphosphatase activity and ultrastructure of lung and kidney associated with oxidative stress induced by acute ethanol intoxication. *Chest*; **121**:589–596.

Sadeh A. (1997). Sleep and melatonin in infants: a preliminary study. *Sleep*, **20**(3):185–191.

Seitz H.K, Egerer G, Simanowski U.A, Waldherr R, Eckey R, Agarwal D.P, Goedde H.W. (1993). Human gastric alcohol dehydrogenase activity: effect of age, sex, and alcoholism. *Gut*, 34.

Shabanzadeh D.M and Novovic S. (2017). Alcohol, smoking and benign hepato biliary disease. *Best Pract Res Clin Gastroenterol*, **31**(5):519– 527

Simonneaux V and Ribelayga C. (2003). Generation of the melatonin endocrine message in mammals: A review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. *Pharmacol. Rev*, **55**:325–395.

Sun A.Y, Ingelman-Sundberg M, Neve E. (2001). Ethanol and oxidative stress. *Alcohol Clin Exp Res*; **25**(5):237–243.

Unalp-Arida A and Ruhl C.E. (2023). Increasing gallstone disease prevalence and associations with gallbladder and biliary tract mortality in the US. *Hepatology*, **77**(6):1882–1895

World Health Organization. (2018). Global status report on alcohol and health 2018. Geneva: World Health Organization.

Yadav D and Lowenfels A.B. (2013). The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology*, **144**(6):1252–1261.

You M and Crabb D.W. (2004). Recent advances in alcoholic liver disease II. Minireview: Molecular mechanisms of alcoholic fatty liver. *Am J Physiol Gastrointest Liver Physiol*; **6**:1–6.

Zawilska J.B, Skene D.J, Arendt J. (2009). Physiology and pharmacology of melatonin in relation to biological rhythms.

Zuiderma G. (1995). Shackelford's Surgery of the Alimentary Tract, 4th ed. Philadelphia, WB Saunders.