INVESTIGATING THE EFFECT OF MELATONIN ON ALCOHOL INDUCED DUODENAL TOXICITY ON WISTAR RATS

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANATOMY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA. IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc) IN ANATOMY.

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NOVEMBER 2025

DECLARARATION

I hereby declare that the project work entitled “I**NVESTIGATING THE EFFECT OF MELATONIN ON ALCOHOL INDUCED DUODENAL TOXICITY ON WISTAR RATS”** submitted to the department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria, is a record of an original work done by me under the guidance of Dr. Edobor Obayuwana, my project supervisor; and this project is submitted in the partial fulfillment of the requirements for the award of the degree of Bachelor of Science in Anatomy. The results embodied in this study have not been submitted to any other university or institute for the award of any degree or diploma.

Signature………………………...

Name……………………………

Guardian………………………...

CERTIFICATION

This is to certify that this project work titled **INVESTIGATING THE EFFECTS OF MELATONIN ON ALCOHOL INDUCED DUODENAL TOXICITY IN WISTAR RATS** was carried out by **DIM JENNIFER NJIDEKA** with matriculation number BMS2101316, of the Department of Anatomy, School of Basic Medical Science, University of Benin City, Edo State, Nigeria.

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

DEDICATION

This work is dedicated to God Almighty, to the loving memory of my parents **JAMES UMEZIKADIM, AND THERESA UMEZIKADIM**, whose love, strength and unwavering faith continue to inspire me. Their memory remains a guiding light in all that I do.

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I thank God Almighty, above all things for the gift of life and the grace he gave me to do this work.

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ABSTRACT

Alcohol (ethanol) is a widely consumed psychoactive substance known to induce oxidative stress and gastrointestinal mucosal damage, particularly in the duodenum. Chronic alcohol exposure generates reactive oxygen species (ROS) and disrupts mucosal integrity. Melatonin, a neurohormone synthesized in the pineal gland and gastrointestinal tract, exhibits antioxidant and anti-inflammatory properties, and may offer protection against alcohol-induced tissue injury. To Investigate the Effects of Melatonin on Alcohol induced duodenal Toxicity in Adult Wistar Rats. Twenty adult male Wistar rats were randomly divided into four groups (n=5): control, alcohol only (50% ethanol), melatonin only (5 mg/kg), and melatonin plus alcohol. All treatments were administered orally via gavage for 28 days. After the exposure period, blood samples were collected to assess oxidative stress markers, and duodenal tissues were harvested for histopathological analysis. Statistical analysis were performed using one-way ANOVA with significance set at $p<0.05$. Alcohol induced ulcer in the mucosa of the duodenum and the ulcer induced was a funnel shaped. The group given melatonin and alcohol showed protective effect, preventing alcohol induced ulceration. In conclusion, melatonin at 5mg/kg prevented against alcohol-induced duodenal ulceration in wistar rats.

CHAPTER ONE

1.1 INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a pleiotropic neurohormone synthesized mainly by the pineal gland (Smith, 2024), with additional production occurring in the gastrointestinal tract, retina, bone maow, and other tissues. Beyond its well-known role in regulating circadian rhythms, melatonin has emerged as a potent antioxidant, anti-inflammatory, immunomodulatory, and oncostatic agent (Nabavi *et al*., 2019). These properties are especially relevant to gastrointestinal physiology, where oxidative stress is implicated in various pathology including peptic ulcer disease, mucous inflammation, and alcohol-induced damage.

Alcohol (ethanol) is a widely consumed psychoactive substance, socially accepted in many cultures, yet associated with a host of health problems when consumed excessively (Tedor, 2021). Upon ingestion, ethanol is rapidly absorbed in the gastrointestinal tract and metabolized in the liver via alcohol dehydrogenase to acetaldehyde, a highly reactive compound that generates reactive oxygen species (ROS) and contributes to cellular damage (Barile, 2019). Chronic alcohol consumption has been linked to oxidative stress, mucosal erosion (Bhattacharyya *et al*., 2014), inflammation (Obayuwana *et al.*, 2024), and disruption of gut barrier function (Kuo, 2024). The duodenum, being the first part of the small intestine that receives chyme from the stomach, is particularly vulnerable to alcohol-induced toxicity due to its role in digestion, nutrient absorption, and proximity to alcohol absorption sites (Caputo *et al*., 2024).

Recent studies have demonstrated that melatonin is produced in much higher concentrations in the gastrointestinal tract than in the pineal gland, and this endogenous melatonin may play a crucial role in maintaining mucosal integrity (Hardeland, 2017). Melatonin acts as a direct scavenger of ROS and indirectly boosts the expression of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Hacışevki and Baba, 2018). Moreover, it modulates inflammatory signaling pathways, including nuclear factor-kappa B (NF-κB) (Kumar *et al*., 2021), which is often activated during alcohol-induced tissue injury.

Animal studies have shown that melatonin may prevent or reverse oxidative and histological damage induced by heavy metals and toxic substances in the stomach, liver, and duodenum (Pal *et al*., 2019). However, there is still a paucity of experimental data specifically addressing the protective effects of melatonin on alcohol-induced duodenal injury. Given the rising incidence of alcohol-related gastrointestinal diseases and the need for safe therapeutic alternatives, investigating melatonin protective role could offer promising insights for transitional applications.

1.1 Statement of the Research 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.

1.2 Justification of Study

Alcohol-induced gastrointestinal disorders, particularly in the duodenum, are of increasing concern due to lifestyle habits and rising alcohol misuse. Melatonin, with its antioxidant and cytoprotective effects, may offer a novel therapeutic approach. Exploring its potential to mitigate alcohol-induced duodenal damage could pave the way for developing safer preventive or curative strategies against GIT toxicity.

1.3 Aim of Study

This study aims to investigate the effects of melatonin on alcohol-induced duodenal toxicity in adult male wistar rats.

1.4 Specific Objectives

The study will investigate the following

- The effect of melatonin and alcohol on the body weight of the Wistar rats
- The effect of melatonin and alcohol on the oxidative stress markers
- The protective role of melatonin on the duodenal histology in alcohol treated rat

CHAPTER TWO

LITERATURE REVIEW

2.1 Melatonin

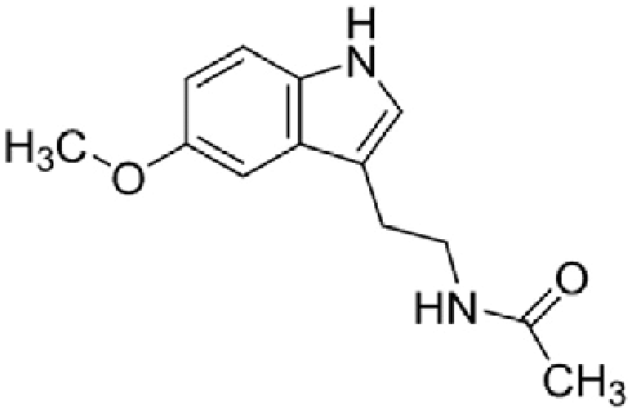

Fig. 2.1 Structure of melatonin

2.1.1 History and Etymology

The study of melatonin began over a century ago. In 1917, researchers Carey P. McCord and Floyd P. Allen discovered that extracts from cow (bovine) pineal glands caused the skin of tadpoles to become lighter. This occurred because the extracts triggered the aggregation of melanin granules within the animals' pigment cells (melanophores) (McCord & Allen, 1917).

It was not until 1958 that the mystery compound was isolated. At Yale University, Aaron B. Lerner and his colleagues extracted and characterized a hormone from bovine pineal glands, which they named melatonin—chemically known as N-acetyl-5-methoxytryptamine (Lerner, Case, Takahashi, Lee, & Mori, 1958).

Shortly after its discovery, scientists observed that melatonin levels fluctuate throughout the dayrising at night and falling in daylight. This finding revealed that melatonin plays a crucial role in regulating the circadian rhythm, the body's internal biological clock (Lerner, Case, & Heinzelman, 1959; Arendt, 2005).

Modern research has revealed that melatonin is an ancient and evolutionarily conserved molecule, present not only in animals but also in bacteria, cyanobacteria, algae, and plants (Tan et al., 2013; Reiter et al., 2014).

Its earliest function appears to have been antioxidant defense, acting as a free-radical scavenger to protect cells from oxidative stress rather than serving as a sleep-inducing hormone (Reiter et al., 2014)

Over time, as multicellular organisms evolved, melatonin acquired additional functions helping to regulate sleep-wake cycles, seasonal reproduction, immune activity, and other physiological processes (Hardeland, 2019).

The name melatonin is derived from the Greek root melas, meaning black or dark, combined with the suffix, tonin, adapted from the word serotonin.

Thus, the word essentially means the dark hormone related to serotonin.

2.1.2 Production

Melatonin (N-acetyl-5-methoxytryptamine) is a neuroendocrine hormone mainly synthesized by the pineal gland, a small, pine-cone-shaped structure located deep within the brain between the two cerebral hemispheres. Although the pineal gland produces the majority of circulating melatonin, smaller amounts are also generated in other tissues such as the retina, gastrointestinal tract, skin, bone marrow, and immune cells (Tan et al., 2013; Reiter et al., 2014). These extra-pineal sites often use melatonin locally, acting through autocrine and paracrine mechanisms.

2.1.3 Metabolism

After being secreted by the pineal gland or administered externally, melatonin rapidly enters the bloodstream, where it undergoes extensive metabolism. The liver is the primary site of this process, although the kidneys and other tissues also contribute to its breakdown (Chong et al., 2015). Melatonin exhibits variable oral bioavailability—ranging from roughly 10% to 50% and has a short half-life of about 20 to 60 minutes in humans. This short duration means its physiological effects are often brief, and its metabolites continue to exert biological activity even after the parent compound is cleared.

2.1.4 Hepatic (Cytochrome P450) Hydroxylation and Conjugation

The main metabolic route of melatonin occurs in the liver, where cytochrome P450 enzymes—especially CYP1A2—hydroxylate it at the 6-position, forming 6-hydroxymelatonin. Other cytochrome isoforms may also participate to a lesser extent (Hardeland et al., 2015). The newly formed compound is then conjugated with either sulfate or glucuronic acid, making it more water-soluble for urinary excretion. This pathway is considered the classical detoxification mechanism for circulating melatonin in mammals.

2.1.5 Kynuramine Pathway (Oxidative and Radical-Mediated Metabolism)

A second, important metabolic route is the oxidative kynuramine pathway, in which melatonin undergoes enzymatic or non-enzymatic oxidation to produce N-acetyl-N-formyl-5-methoxykynuramine (AFMK) and its subsequent derivative, N-acetyl-5-methoxykynuramine (AMK) (Tan et al., 2007).

Melatonin can also react with reactive oxygen species (ROS) or undergo peroxidase-mediated reactions, generating intermediates such as 3-hydroxymelatonin, which are later converted into AFMK and AMK (Hardeland et al., 2015). These metabolites are biologically active and extend melatonin’s antioxidant and anti-inflammatory effects beyond its initial presence in the bloodstream.

2.1.5.1 Physiological Function

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine hormone primarily secreted by the pineal gland, though it is also synthesized in several peripheral tissues including the retina, gastrointestinal tract, skin, bone marrow, and lymphocytes (Reiter et al., 2014; Hardeland et al., 2011). It acts through endocrine and paracrine pathways, binding mainly to melatonin receptors (MT_1 and MT_2) that are widely distributed throughout the body (Dubocovich et al., 2010).

Melatonin performs numerous physiological roles, particularly in regulating circadian rhythms and sleep, and in maintaining antioxidant defense, immune function, and neuroendocrine balance.

2.1.5.2 Regulation of Circadian Rhythm and Sleep

Melatonin well known functions is to control the circadian rhythm, the body's internal 24-hour clock. The suprachiasmatic nucleus (SCN) of the hypothalamus acts as the main circadian pacemaker, receiving light cues from the retina and directing pineal melatonin secretion (Arendt, 2006).

Melatonin levels rise at night and fall during daylight, signaling the onset of biological night (Reiter et al., 2014). By interacting with MT_1 and MT_2 receptors in the SCN, melatonin helps regulate the sleep–wake cycle, encourages sleep onset, and improves sleep quality (Hardeland et al., 2011; Pandi-Perumal et al., 2006).

MT_1 receptors promote sleep initiation by reducing SCN neuronal activity.

MT_2 receptors help adjust and synchronize circadian phases (Dubocovich et al., 2010).

2.1.5.3 Antioxidant and Free Radical Scavenging Effects

Melatonin functions as a powerful antioxidant, directly neutralizing reactive oxygen and nitrogen species while also enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (Tan et al., 2002).

It safeguards mitochondrial membranes, lipids, and DNA from oxidative damage (Reiter et al., 2014). Because melatonin is both fat- and water-soluble, it readily crosses cell membranes and the blood–brain barrier, giving it a key role in neuroprotection (Tan et al., 2007).

2.1.5.4 Immunomodulatory Role

Melatonin significantly influences immune regulation. It enhances cytokine production, stimulates natural killer (NK) cells, and promotes T-lymphocyte maturation (Carrillo-Vico et al., 2005).

Additionally, it maintains the balance between pro- and anti-inflammatory responses, reducing oxidative stress and tissue injury during inflammation or infection (Mayo et al., 2005). The presence of melatonin receptors on macrophages and lymphocytes allows for autocrine and paracrine immune modulation.

2.1.6 Reproduction and Endocrine Functions

Melatonin affects reproductive hormones by modulating gonadotropin-releasing hormone (GnRH) in the hypothalamus, which influences luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release from the pituitary (Reiter et al., 2009).

In seasonal breeders, it conveys photoperiodic information, linking day length to reproductive timing (Arendt, 2006). In humans, it contributes to puberty regulation, menstrual cycling, and ovarian function.

Beyond reproduction, melatonin interacts with metabolic and endocrine systems, influencing insulin secretion, thyroid hormone production, and adrenal cortisol rhythms (Hardeland et al., 2011).

2.1.7 Neuroprotective Effects

Melatonin offers protection to the nervous system through its antioxidant, anti-inflammatory, and anti-apoptotic activities (Reiter et al., 2010). It has shown beneficial effects in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, and in ischemic brain injury (Pandi-Perumal et al., 2006).

By maintaining mitochondrial function and reducing excitotoxicity, melatonin supports neuronal survival and cognitive performance (Tan et al., 2007).

2.1.8 Cardiovascular Protection

Melatonin has a protective effect on the cardiovascular system. It regulates blood pressure, vascular tone, and heart rhythm (Simko & Paulis, 2007). Its antioxidant and anti-inflammatory actions help prevent atherosclerosis and ischemic heart damage.

observations suggest that melatonin can lower nocturnal blood pressure, showing it's role in managing hypertension (Reiter et al., 2010).

2.1.9 Gastrointestinal and Peripheral Actions

Large quantities of melatonin are produced in the gastrointestinal tract, where it assists in motility, mucosal protection, and acid secretion regulation (Bubenik, 2002). It also exhibits anti-ulcer and anti-inflammatory effects, improving intestinal integrity.

Other peripheral actions include stimulating bone formation, influencing skin pigmentation, and exerting anticancer properties by inhibiting tumor growth and promoting apoptosis (Hardeland et al., 2011).

2.1.9.1 Animal and Human foods

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone best known for its role in regulating sleep–wake cycles, but it is also widely present in foods from both animal and plant origins. Consuming melatonin-rich foods can increase blood melatonin levels, strengthen antioxidant defense systems, and support normal sleep and metabolism (Reiter et al., 2014; Tan et al., 2014)

Besides being produced in the pineal gland, melatonin occurs naturally in various foods. Its amount varies with species, environmental conditions, and processing methods (Kłysik et al., 2020). Once ingested, dietary melatonin is absorbed through the gastrointestinal tract, passes the blood–brain barrier, and can actively influence physiological processes (Saadat et al., 2021).

2.1.9.2 Chemical feedstock

Melatonin (N-acetyl-5-methoxytryptamine) is a small hormone belonging to the indoleamine family. It is produced from the essential amino acid tryptophan, which acts as the main starting material, or chemical feedstock, for its synthesis. The production of melatonin takes place through a sequence of enzyme-controlled reactions, where each step converts one compound into another until melatonin is ultimately formed (Reiter et al., 2014; Hardeland et al., 2011).

2.1.9.3 Laboratory Research

Table 2.1 Food sources of melatonin

Research Focus	Food Sources
Melatonin in food	Cereals (rice, oats, barley, corn, wheat)
Melatonin in vegetables	Tomatoes, pepper, mushroom, corn
Melatonin in Fruits	Banana, pineapple, cherries, grapes oranges
Melatonin in Beverages	Wine, beer, coffee, tea
Melatonin in Animals	Cow's milk, egg, fish, meat

2.2 ALCOHOL

Fig 2.2 Absolute alcohol

2.2.1 DEFINITION

Absolute alcohol, also called anhydrous ethanol or 100% pure ethanol, is ethanol that contains less than 1% water by weight (McDonnell et al., 2012). It represents the purest form of ethanol that can be obtained under normal laboratory conditions. This form is different from ordinary ethanol or rectified spirit, which typically has about 95% ethanol and 5% water. The reason it cannot be distilled further by simple methods is the formation of an azeotrope at that concentration (Pavia et al., 2015).

The chemical formula for ethanol, including absolute alcohol, is C_2H_5OH. It is a colorless, volatile, and flammable liquid with a distinct alcoholic odor. Absolute alcohol boils at around 78.37°C and has a density of 0.789 g/mL at 20°C (Lide, 2004). Because it contains almost no water, it has strong solvent and dehydrating properties, making it very useful for chemical reactions that are sensitive to moisture.

Alcohol stretches back thousands of years and is deeply tied to human history and culture. The term “alcohol” comes from the Arabic word al-kuḥl, which originally meant a fine powder of antimony used in cosmetics. Over time, the word came to refer to any distilled substance and eventually to ethanol, the intoxicating element found in alcoholic beverages (Forbes, 1953; Unger, 2004).

Alcoholic drinks were probably discovered by accident when early humans noticed that fruits left to ferment naturally produced a pleasant and intoxicating liquid. Archaeological discoveries show that people were deliberately fermenting drinks as early as 7000–6600 BCE in Neolithic China, where pottery jars contained residues of fermented rice, honey, and fruit (McGovern et al., 2004). Other early evidence includes wine-making in Georgia around 6000 BCE and beer brewing in Mesopotamia and Egypt around 4000 BCE (Hornsey, 2003).

2.2.2 STRUCTURAL FORMULA

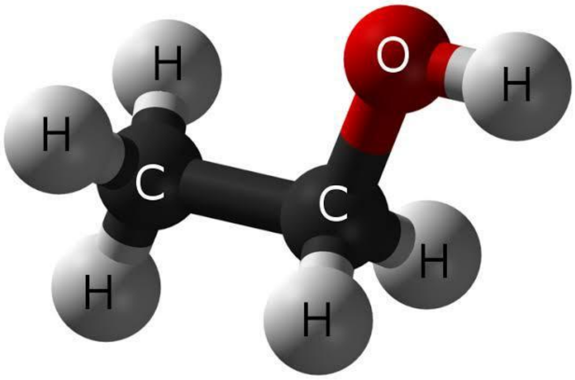

Fig. 2.3 Structural formula of melatonin

2.2.3 CHARACTERISTICS

2.2.3.1 Physical Properties

Lower alcohols, such as methanol, ethanol, and propanol, are colorless liquids at room temperature. They have a distinctive odor and a slightly burning taste. (Morrison & Boyd, 2011). Alcohols dissolve easily in water, especially those with short carbon chains (one to three carbon atoms). This happens because the hydroxyl group (–OH) can form hydrogen bonds with water molecules. However, as the carbon chain grows longer, the nonpolar portion becomes dominant, reducing solubility (McMurry, 2014). The boiling points of alcohols are significantly higher than those of hydrocarbons or ethers with similar molecular weights. This is mainly due to the presence of hydrogen bonding between alcohol molecules. (Solomons & Fryhle, 2016). Alcohols are generally denser and more viscous than comparable hydrocarbons because of hydrogen bonding between molecules. The density of ethanol is about 0.789 g/cm^3 at 20°C, and viscosity tends to increase with the length of the carbon chain (Morrison & Boyd, 2011).

2.2.3.2 Chemical Properties

The chemical behavior of alcohols is mainly determined by their hydroxyl group (–OH) and the carbon skeleton attached to it. Alcohols can undergo a wide range of reactions such as oxidation, dehydration, esterification, and substitution.

Alcohols act as weak acids. They can donate a hydrogen ion (H^+) from their hydroxyl group, forming alkoxide ions (RO^-). Their acidity is slightly greater than that of water in some cases but far less than that of carboxylic acids. Acidity generally decreases as the carbon chain gets longer (McMurry, 2014).

2.2.3.3 Biological role and Research

Ethanol is mainly broken down in the liver through two key enzymes: alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). These convert ethanol first into acetaldehyde, a toxic intermediate, and then into acetic acid, which enters the Krebs (citric acid) cycle to produce energy (Cederbaum, 2012). It's acts as a depressant on the central nervous system. It enhances the action of GABA, the brain's main inhibitory neurotransmitter, while reducing glutamate activity. This leads to relaxation, reduced anxiety, and euphoria at low doses. Higher doses, however, impair movement, judgment, and can cause unconsciousness or respiratory depression (Koob & Volkow, 2010). Light or moderate alcohol consumption has been shown to increase HDL (good) cholesterol, reduce blood clot formation, and lower the risk of certain heart diseases (Brien et al., 2011). On the other hand, heavy or long-term drinking can cause high blood pressure, heart muscle damage, and hormonal disturbances such as reduced testosterone and estrogen imbalance (O'Keefe et al., 2007).

Research focuses on how alcohol metabolism contributes to liver injury, mainly through the buildup of acetaldehyde and free radicals (ROS). Work by Lieber (1998) and Cederbaum (2012) found that chronic alcohol use triggers the microsomal ethanol-oxidizing system

(MEOS), particularly the enz yme CYP2E1, which increases oxidative stress and causes liver cell damage.

Neuroscience research using brain images have shown that alcohol disrupts dopamine signaling in the brain’s reward system. This leads to dependence, tolerance, and withdrawal symptoms when drinking stops (Koob & Volkow, 2010). These studies have deepened understanding of addiction mechanisms.

Population studies have explored both positive and negative effects of alcohol use. Moderate drinking can lower the risk of coronary heart disease, while excessive intake is strongly associated with liver cirrhosis, certain cancers, and neurological disorders (Brien et al., 2011; Rehm et al., 2009).

Ethanol is used in drug formulations as a solvent and preservative, as well as in laboratory assays. It helps dissolve compounds that are not water-soluble and is also used to extract active ingredients from plants for herbal medicine research (Faiq et al., 2017).

2.2.4 USES OF ALCOHOL

It can be used for the following

- Medicinal and Pharmaceutical uses (Trease & Evans, 2009).
- Industrial uses (Carey & Giuliano, 2013).
- Laboratory and Scientific uses (Bancroft & Gamble, 2008).
- Food and Beverage Industries (O’Keefe et al., 2007).
- Domestic and Cosmetic uses (Solomons & Fryhle, 2016).

2.3 ORGAN OF STUDY: THE DUODENUM

The duodenum is the first and shortest section of the small intestine, measuring about 25–30 cm in length, and it plays a vital role in both digestion and nutrient absorption (Standring, 2021). It extends from the pylorus of the stomach to the duodenojejunal flexure, where it continues as the jejunum. Structurally, the duodenum is divided into four parts — the superior (first), descending (second), horizontal (third), and ascending (fourth) segments (Moore et al., 2023)

The duodenum curves around the head of the pancreas, forming a characteristic C-shape. The descending portion receives secretions from the common bile duct and pancreatic duct, which unite to form the hepatopancreatic ampulla (of Vater), controlled by the sphincter of Oddi (Drake et al., 2023). Its blood supply comes from branches of both the celiac trunk (via the superior pancreaticoduodenal artery) and the superior mesenteric artery (via the inferior pancreaticoduodenal artery). This dual supply reflects its embryological link to both the foregut and midgut. Venous blood drains into the portal vein, following the same route as the arteries.

2.3.1 STRUCTURE OF THE DUODENUM

The duodenum is a C-shaped organ that receives partially digested food from the stomach.

It is connected to the stomach by the pyloric sphincter and to the jejunum by the duodenojejunal flexure.

The duodenum is where digestive juices from the pancreas and bile from the gallbladder and liver enter to help with digestion and nutrient absorption.

It is divided into four main sections: the superior, descending, transverse, and ascending regions.

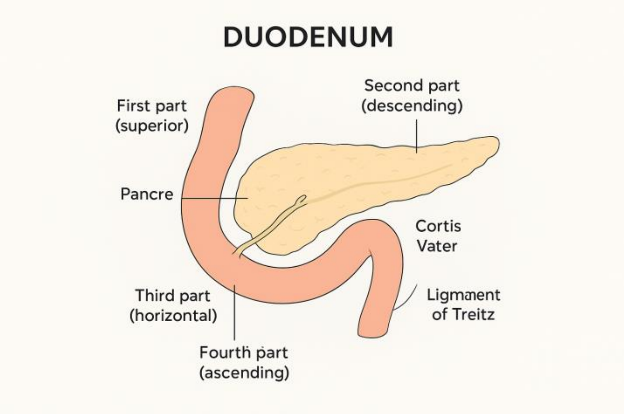

Fig. 2.4: DUODENUM

2.3.2 EMBRYOLOGY OF THE DUODENUM

The duodenum develops from the end of the foregut and the beginning of the midgut, which makes it a transitional organ in both structure and function. Its development determines its final shape, position, blood supply, and its close relationship with the pancreas and bile ducts. Any disturbance during this process can lead to congenital defects such as duodenal atresia or stenosis, annular pancreas, or malrotation-related obstructions (Sadler, 2021; Moore et al., 2023)

During the third and fourth weeks of development, the embryo folds to form the primitive gut tube, which is lined by endoderm. This tube is divided into the foregut, midgut, and hindgut. The portion that later forms the duodenum lies between the lower part of the foregut and the upper part of the midgut. The junction between these regions corresponds roughly to where the bile duct joins the developing intestine (Sadler, 2021).

As the embryo grows, the duodenal region elongates and rotates, forming the characteristic C-shaped curve that wraps around the developing pancreas. The pancreas develops from two buds (a dorsal and a ventral bud) that arise from the duodenal wall. During rotation, the ventral pancreatic bud moves around to the back of the duodenum to fuse with the dorsal bud, forming the head and uncinate process of the pancreas. This close relationship explains why diseases of the pancreas often affect the duodenum and vice versa (Moore et al., 2023; Standring, 2021).

The inner lining of the duodenum is made up of rapidly growing epithelial cells, temporarily blocks the lumen. Later, vacuoles (small spaces) appear in the epithelial plug, merging to restore the lumen . If this process fails, the duodenum may remain blocked (atresia) or become narrowed (stenosis). These conditions can cause vomiting in newborns and are often recognized by the double bubble sign on X-rays (Sadler, 2021).

2.3.2 ANATOMICAL DIVISIONS OF DUODENUM

Anatomically, the duodenum is divided into four parts, each with distinct positions, relationships (Moore et al., 2023).

- First Part (Superior Part)

This section runs from the pylorus to the neck of the gallbladder, about 5 cm long, and lies at the level of the first lumbar vertebra (L1) (Drake et al., 2023). It lies anteriorly to the liver and gallbladder, while posteriorly it touches the portal vein, common bile duct, and gastroduodenal artery. it has a smooth inner lining. Because of its proximity to acidic gastric contents, this part is the most common site for duodenal ulcers (Standring, 2021).

- Second Part (Descending Part)

This portion descends along the right side of the vertebral column, from L1 to L3, and measures 7–10 cm in length (Moore et al., 2023). Anteriorly, it is in contact with the right lobe of the liver, transverse colon, and small intestine loops, while posteriorly, it lies over the right kidney and ureter. The bile duct and main pancreatic duct join to form the hepatopancreatic ampulla (of Vater), which opens into the duodenum at the major duodenal papilla. The accessory pancreatic duct, when present, opens at the minor duodenal papilla just above it (Standring, 2021).

- Third Part (Horizontal or Inferior Part)

This part crosses the midline, running horizontally from right to left at the level of L3, and measures about 8–10 cm (Standring, 2021). It passes in front of the aorta and inferior vena cava, and is crossed anteriorly by the superior mesenteric artery (SMA) and vein.

- Fourth Part (Ascending Part)

This short segment, roughly 2.5 cm long, ascends on the left side of the aorta up to the L2 level, ending at the duodenojejunal flexure (Drake et al., 2023).

2.3.3 LIGAMENTS

Ligaments of the Duodenum

- **Suspensory Ligament of the Duodenum**

The ligament of Treitz, or suspensory muscle of the duodenum, is a fibromuscular band that connects the duodenojejunal flexure to the right crus of the diaphragm (Moore et al., 2022). It originates near the celiac trunk and the left crus of the diaphragm, passes behind the pancreas, and attaches to the junction where the duodenum becomes the jejunum. This ligament is made up of both skeletal muscle fibers from the diaphragm (upper part) and smooth muscle fibers from the duodenal wall (lower part) (Drake et al., 2019). Because of this combination, movements of both the diaphragm and the intestine can affect the angle of the duodenojejunal junction. The ligament of Treitz helps to maintain the proper angle of the duodenojejunal flexure, ensuring smooth passage of intestinal contents. It also acts as a supporting structure that keeps the junction fixed in position.

- **Hepatoduodenal Ligament**

The hepatoduodenal ligament forms the right free border of the lesser omentum, stretching from the porta hepatis of the liver to the first part of the duodenum (Moore et al., 2022). It encloses critical structures known collectively as the portal triad. The ligament carries the following structures:, Hepatic artery proper, Common bile duct, Portal vein. It may also contain lymphatic vessels and autonomic nerve fibers (Standring, 2021). The hepatoduodenal ligament links the liver and duodenum, while transmitting major blood vessels, bile ducts, and nerves between them. It also helps form the anterior boundary of the epiploic (Winslow's) foramen, which connects the lesser and greater sacs of the peritoneum (Drake et al., 2019).

It serves as an essential surgical reference during operations like cholecystectomy or portal triad clamping (Pringle maneuver) to control bleeding.

- **Duodenocolic Ligament**

The duodenocolic ligament is a peritoneal fold that connects the ascending part of the duodenum to the transverse colon (Moore et al., 2022). It stabilizes the relative positions of these two organs within the abdomen. This ligament helps anchor and support the duodenojejunal flexure. It also indirectly influences intestinal movement and peristalsis by maintaining tension among the surrounding mesenteric structures (Standring, 2021). Inflammation or adhesion involving the duodenocolic ligament may lead to intestinal obstruction, malrotation, or even internal herniation (Mishalany et al., 2018).

2.3.4 BLOOD SUPPLY

The arterial supply of the duodenum corresponds to its embryological origin, which includes both foregut and midgut components.

The upper portion of the duodenum, develops from the foregut and is supplied primarily by the superior pancreaticoduodenal artery. This artery arises from the gastroduodenal artery, it is a branch of the common hepatic artery (Drake et al., 2020). The superior pancreaticoduodenal artery splits into anterior and posterior branches. These branches run along the front and back surfaces of the duodenum and the head of the pancreas, forming anastomoses (connections) with branches of the inferior pancreaticoduodenal artery (Standring, 2021).

The lower portion of the duodenum arises from the midgut and receives blood from the inferior pancreaticoduodenal artery, a branch of the superior mesenteric artery (Moore et al., 2018). This artery also divides into anterior and posterior branches, which ascend to connect with the branches of the superior pancreaticoduodenal artery. Together, they form a continuous vascular arcade that links the celiac trunk and SMA territories (Gray et al., 2021).

The anterior and posterior pancreaticoduodenal arcades—created by the superior and inferior pancreaticoduodenal arteries—serve as an important collateral network between the celiac and superior mesenteric systems. This connection ensures a steady blood flow to the duodenum and pancreas, even if one of the main arteries is obstructed (Standring, 2021).

The superior pancreaticoduodenal veins drain into the portal vein through the gastroduodenal vein. The inferior pancreaticoduodenal veins empty into the superior mesenteric vein (SMV). Altogether, these veins contribute to the portal venous system, which carries nutrient-rich blood to the liver for further processing (Moore et al., 2018; Drake et al., 2020).

The lymphatic drainage of the duodenum also reflects its dual blood supply, Anterior lymphatic vessels drain into the pancreaticoduodenal and pyloric lymph nodes.

Posterior lymphatic vessels drain into the superior mesenteric and celiac lymph nodes.

From these nodes, lymph ultimately passes to the cisterna chyli via the intestinal trunk, and then enters the thoracic duct (Standring, 2021).

2.3.5 NERVE SUPPLY

The duodenum, which forms the first and shortest section of the small intestine, receives a rich and complex autonomic nerve supply that regulates its motility, secretion, and reflex activities. This neural control is provided by both extrinsic nerves (sympathetic and parasympathetic) and the intrinsic enteric nervous system, which work together to maintain proper digestive function and coordination (Standring, 2021).

2.3.6 HISTOLOGY OF THE DUODENUM

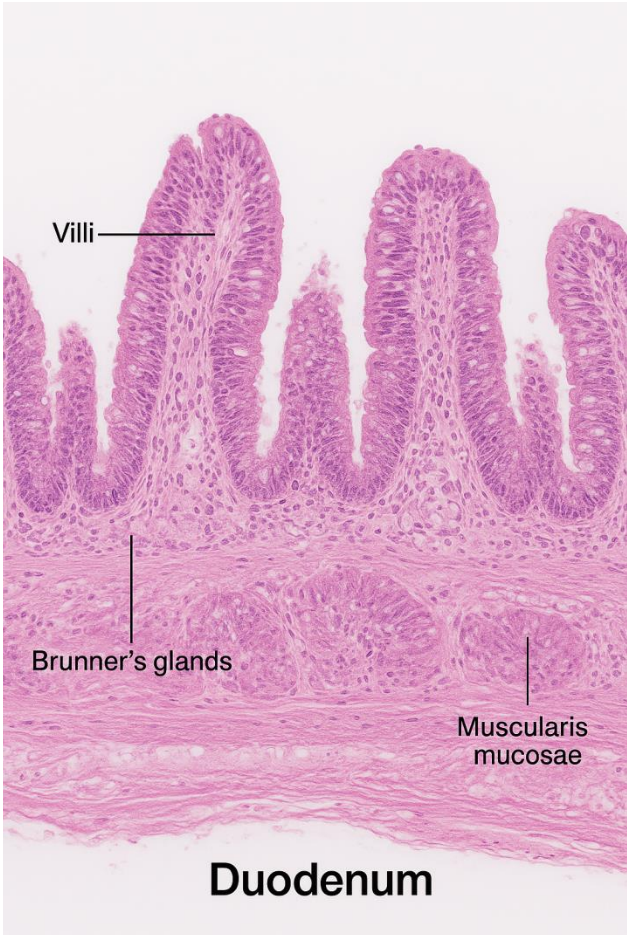

Fig. 2.5: Histological image of the Duodenum

The duodenum is made up of four main layers: the mucosa, submucosa, muscularis externa, and serosa/adventitia (Standring, 2021; Gartner & Hiatt, 2014).

The mucosa forms the innermost lining of the duodenum and is divided into three layers, the epithelium, the lamina propria, and the muscularis mucosae. The epithelial layer is composed of simple columnar cells that contain two main types of cells absorptive cells (enterocytes) and goblet cells. Enterocytes have numerous microvilli that together form the brush border, greatly increasing the surface area for absorption. The brush border also houses enzymes like disaccharidases and peptidases, which help complete the final stages of digestion (Ross & Pawlina, 2020). The goblet cells produce mucus, which coats and protects the duodenal lining from stomach acid and mechanical irritation.

Beneath the epithelium lies the lamina propria, a loose connective tissue layer that contains capillaries, lymphatic vessels (lacteals), and immune cells. Each villus — a finger-like projection of the mucosa has a core made up of this lamina propria. The lacteals absorb fats in the form of chylomicrons, transporting them into the lymphatic system (Gartner & Hiatt, 2014). Embedded within the lamina propria are intestinal glands (crypts of Lieberkühn), which are tubular structures between the villi. These contain stem cells that continually renew the intestinal lining, Paneth cells that secrete lysozyme to fight bacteria, and enteroendocrine cells involved in hormonal control (Ross & Pawlina, 2020).

The muscularis mucosae is a thin layer of smooth muscle fibers that can contract gently, causing the villi to move slightly. This motion improves contact between the intestinal contents and the mucosal surface, enhancing absorption (Standring, 2021).

The submucosa of the duodenum is unique and serves as a key distinguishing feature. It contains Brunner's glands (duodenal glands) — large, branched tubuloalveolar glands found only in the duodenum (Ross & Pawlina, 2020).

Brunner's glands secrete a thick, alkaline mucus rich in bicarbonate ions, which neutralizes stomach acid and protects the duodenal wall. Their secretions also provide the proper pH environment for pancreatic enzymes to act efficiently and prevent the mucosa from damage by digestive juices (Young et al., 2020). Brunner's glands open into the crypts of Lieberkühn and are most abundant in the proximal duodenum, decreasing in number as the intestine continues distally (Moore et al., 2023). The submucosa also contains blood vessels, lymphatics, and the submucosal (Meissner's) nerve plexus, which helps control glandular secretion and local blood flow.

The muscularis externa consists of two smooth muscle layers:

The inner circular layer, which is responsible for segmentation and mixing of intestinal contents.

The outer longitudinal layer, which propels chyme forward by peristalsis.

Between these two layers lies the myenteric (Auerbach's) plexus, part of the enteric nervous system that regulates motility, tone, and coordination of these muscle layers (Furness, 2012). These movements help blend chyme with bile and pancreatic secretions for effective digestion**.**

2.3.7 FUNCTIONS OF THE DUODENUM

The duodenum is the primary site for chemical digestion in the small intestine. It receives chyme—a semi-fluid mixture of food and gastric juice—from the stomach through the pyloric sphincter. This material is then mixed with bile, pancreatic enzymes, and intestinal secretions to complete digestion (Hall & Hall, 2020). Another key function of the duodenum is to neutralize the acidic chyme that enters from the stomach. The Brunner's glands in its submucosa release alkaline mucus rich in bicarbonate, which protects the lining of the duodenum from acid damage and keeps the pH around 7–8—ideal for enzyme activity

(Mescher, 2021; Ross & Pawlina, 2020). The jejunum and ileum do most of the nutrient absorption, and the duodenum still absorbs important minerals and simple nutrients such as iron, calcium, magnesium, and simple sugars (Kierszenbaum & Tres, 2016). Iron is mainly absorbed in the upper part of the duodenum. This process depends on the acidic environment and a special transporter protein known as DMT1 (divalent metal transporter 1) (Andrews, 2020). Calcium absorption depends on vitamin D and occurs through both cellular (transcellular) and between-cell (paracellular) pathways (Guyton & Hall, 2020). The duodenum also has several ways to protect its lining from injury. Its mucus layer and bicarbonate secretion create an alkaline barrier that shields the mucosa from stomach acid. The tight junctions between its epithelial cells also prevent acid from leaking back into the tissue (Ross & Pawlina, 2020).

2.3.8 DISEASE OF THE DUODENUM

- Functional and Mobility disease
- Vascular and ischemia condition
- Malabsorption Disorder
- Obstructure and Structural Disorder
- Neoplastic Disease
- Infectious Disease
- Ulcerative Disease
- Inflammatory Disease.

TREATMENT

- Surgical Treatment
- Antibiotic Use
- Dietary and life style changes

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Distilled water, syringes, weighing scale, chloroform, surgical gloves, cotton wool, beakers, universal bottles, laboratory cages, sawdust, formalin, slides, and paraffin wax, ceramic plates, tissue embedding station, microscope, eosin, paraffin wax, xylene, cotton wool, conical flask, microtome, slide tray, hematoxylin

3.2 Purchase of Melatonin and Alcohol

Melatonin was manufactured by Bactolac pharmaceutical Inc, it was purchased at thelver pharmacy opposite UBTH Benin city, Edo State. Alcohol was purchased at Emmytex Biological stores at 47 new Lagos Road opposite UBTH Benin city, Edo State.

3.3 METHOD

3.3.1 Experimental Animals and Design

Twenty wistar rats of were purchase from the Department of Human Anatomy, University of Benin, Edo State. The animals were kept in the animal holding of the Department of Human Anatomy for two weeks to acclimatize. They were housed in well ventilated aluminium cages at room temperature in hygienic conditions under natural light (13hours) and dark (11 hours) schedule and were fed on standard laboratory diet. Food and water were given ad libitum.

TABLE 1.0 EXPERIMENTAL DESIGN

In this study, the animals were divided into four groups, A, B,C and D with each group having five wistar rats which were weighed prior to the administration. The experimental period spanned for 28 days. The rats were administered with melatonin and alcohol for 28 days. Twenty adult wistar

GROUP	DOSAGE
GROUP A	Served as control and were fed with animal fed and water ad libitun
GEOUP B	Were administered (Melatonin+ Alcohol): 5mg/kg melatonin and 50% alcohol
GROUP C	(Melatonin only) 5mg/kg melatonin
GROUP D	(Alcohol only) 50% absolute alcohol

All treatments will be administered orally via gavage for 28 consecutive days

3.4 Sample Collection and Biochemical Analysis

After 28 days of administration,

- Rats were be weighed and sacrificed under chloroform anesthesia.
- Blood were be collected for oxidative stress marker analysis.
- Duodenum were be harvested and fixed in 10% formal saline.
- Tissue processing were follow standard histopathological protocols, including paraffin embedding, sectioning, staining, and microscopic analysis.

3.6 Statistical Analysis

Data will be analyzed using Graphpad Prism Version . One-way ANOVA and post hoc multiple comparison tests will be used to determine statistical significance, with a p-value < 0.05 considered significant.

CHAPTER FOUR

RESULTS AND STATISTICAL ANALYSIS

Data were analyzed using a Graphpad Prism, One-way ANOVA and post hoc multiple comparison test were used to determine statistical significance with a p-value < 0.05 considered significant.

4.1 Initial and Final body weight

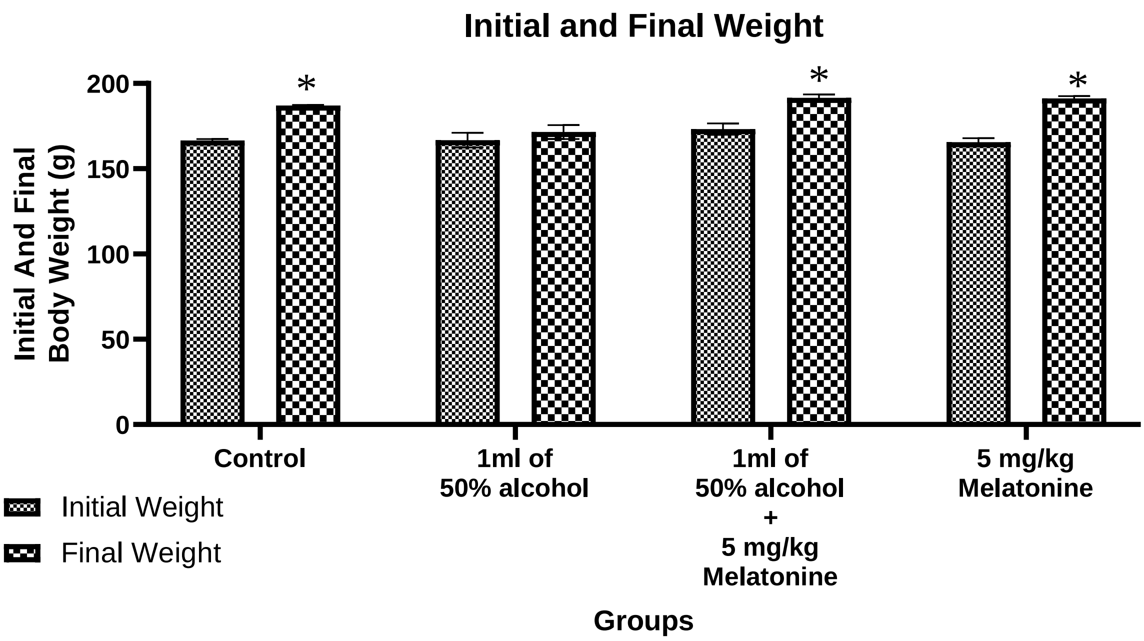

Fig. 1: Initial and Final weight after 28 days of administration Values are given as mean ± SEM.$^{*}p< 0.05$ compared with the initial weight within group.

4.2. Weight Change

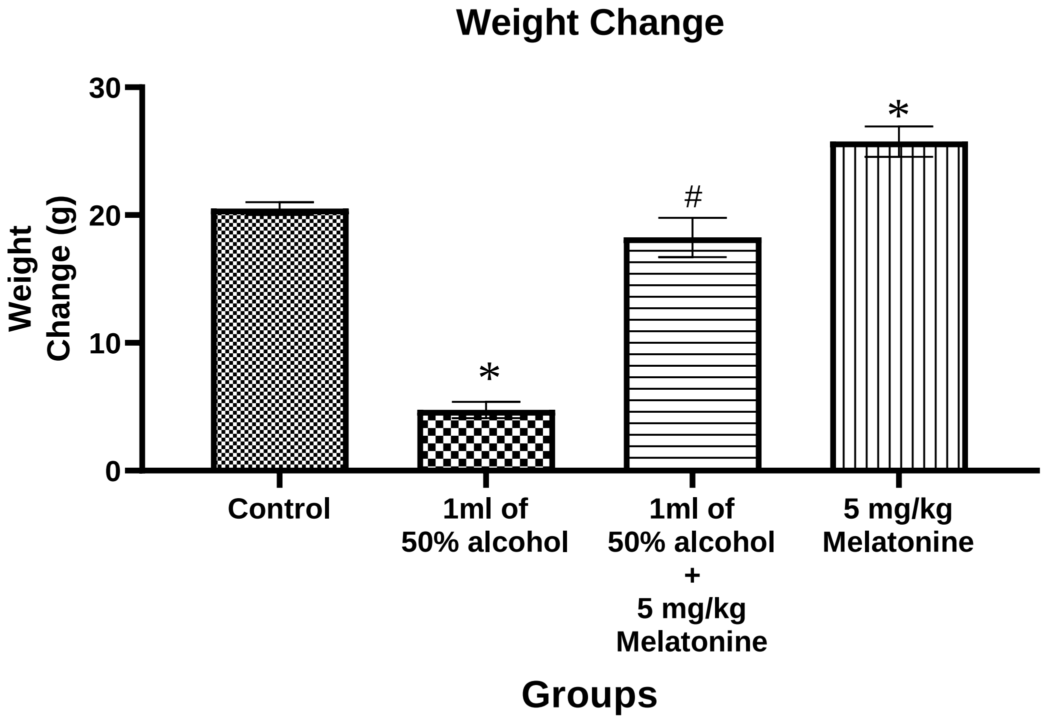

Fig. 2: weight change after 28 days. Values are given as mean ± SEM. *$p < 0.05$ compared with the control group; #$p < 0.05$ compared with the 1ml of 50% alcohol alone group.

4.3 Duodenal Superoxide Dismutase (SOD) level

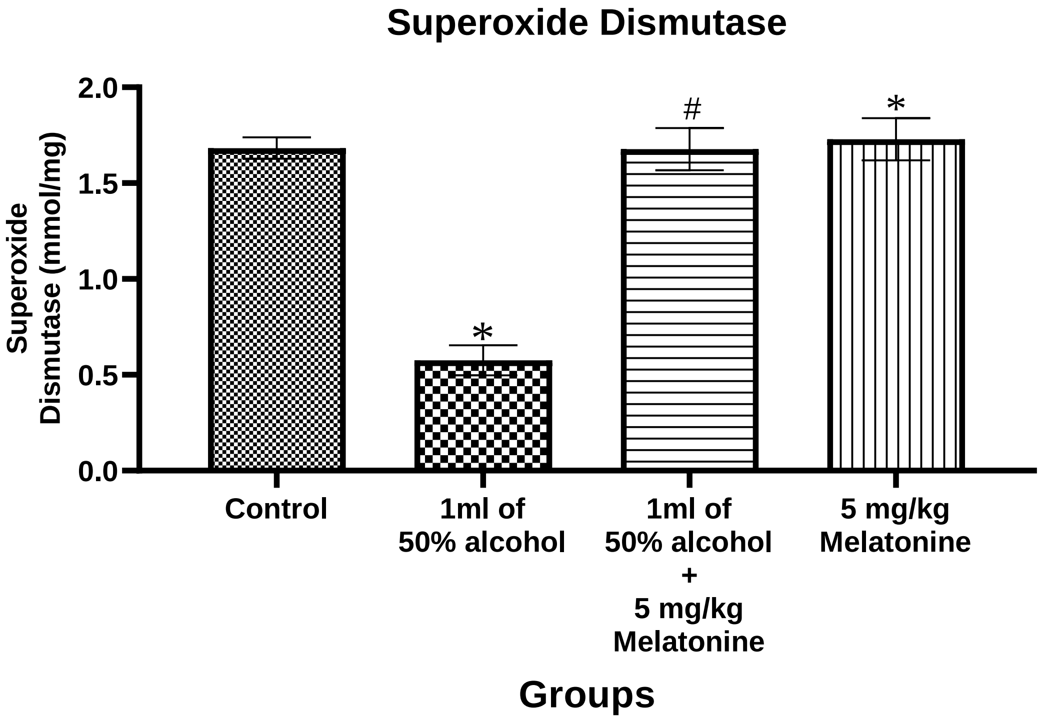

Fig. 3: Superoxide dismutase activity in the Duodenum of control and treatment groups after 28 days. Values are given as mean ± SEM. *$p < 0.05$ compared with the control group; #$p < 0.05$ compared with the 1ml of 50% alcohol alone group.

4.4 Duodenal Catalase enzyme (CAT) level

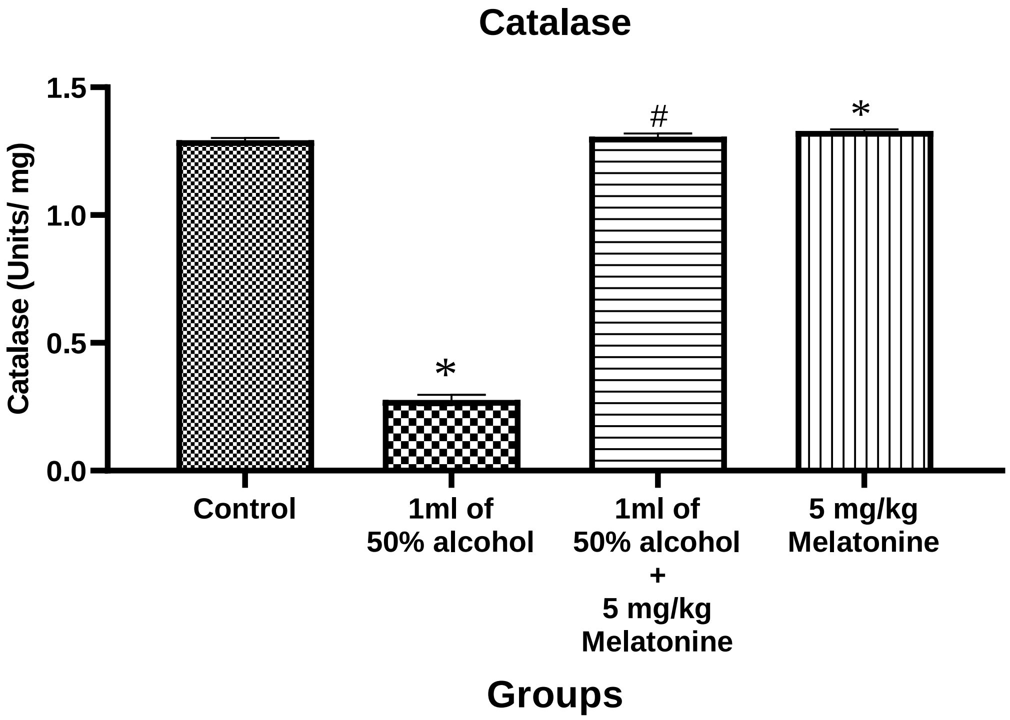

Fig. 4: Catalase activity in the Duodenum of control and treatment groups after 28 days. Values are given as mean ± SEM. *p< 0.05 compared with the control group; #p< 0.05 compared with the 1ml of 50% alcohol alone group.

4.5 Glutathione peroxidase (GPx)

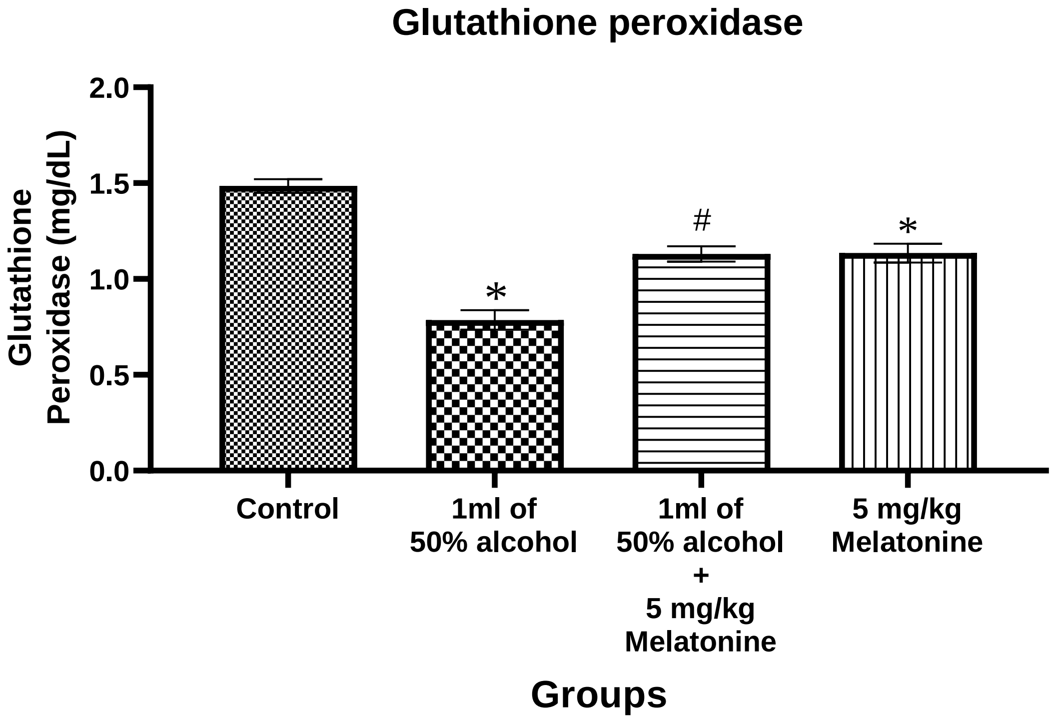

Fig. 5: Glutathione Peroxidase activity in the Duodenum of control and treatment groups after 28 days. Values are given as mean ± SEM. *$p < 0.05$ compared with the control group; #$p < 0.05$ compared with the 1ml of 50% alcohol alone group.

4.6 Malondialdehyde (MDA)

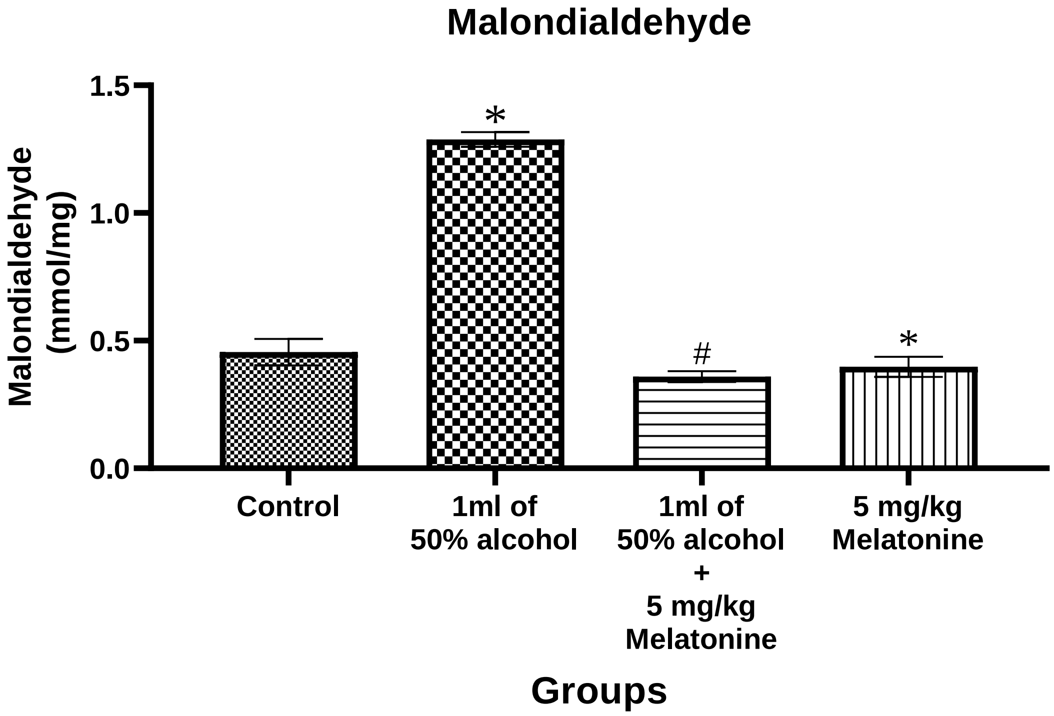

Fig. 6: Lipid peroxidation activity in the Duodenum of control and treatment groups after 28 days. Values are given as mean ± SEM. **p*< 0.05 compared with the control group; #*p*< 0.05 compared with the 1ml of 50% alcohol alone group.

4.7 Histology of the Duodenum

Plate 1. Rat duodenum, control, composed of normal architecture: mucosal villi (MV), mucosal glands (MG), sub mucosa (SM) and muscularis propria (MP): H&E 40 X

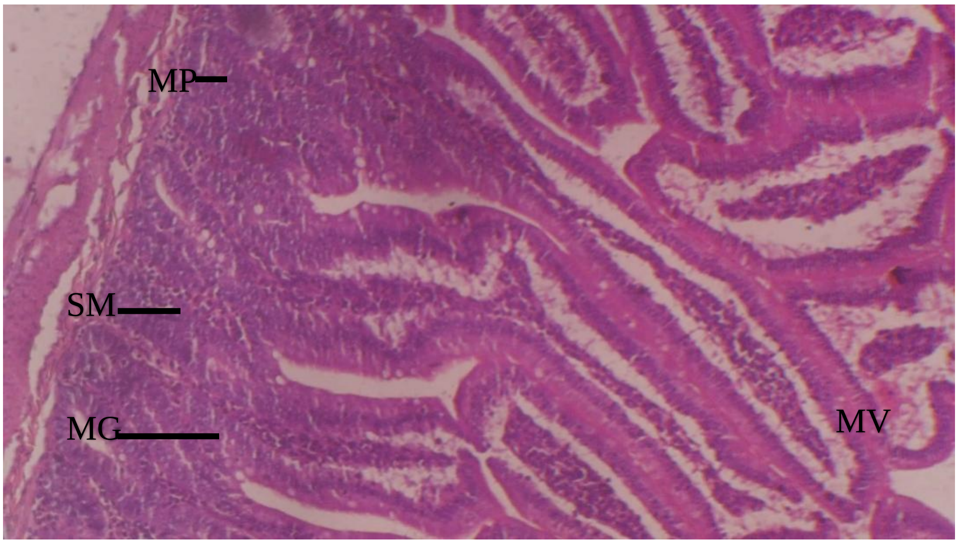

Plate 2. Rat duodenum, control, composed of normal architecture: mucosal villi (MV), mucosal glands (MG), sub mucosa (SM) and muscularis propria (MP) : H&E 100 X

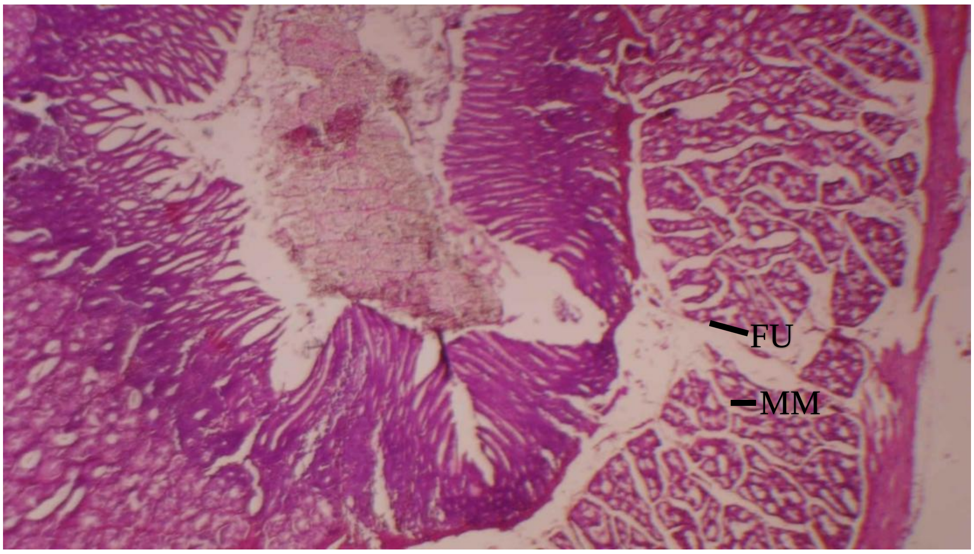

Plate 3. Rat duodenum given Alcohol only show: funnel-shaped ulcer (FU), muscularis mucosa (MM): H&E 40 X

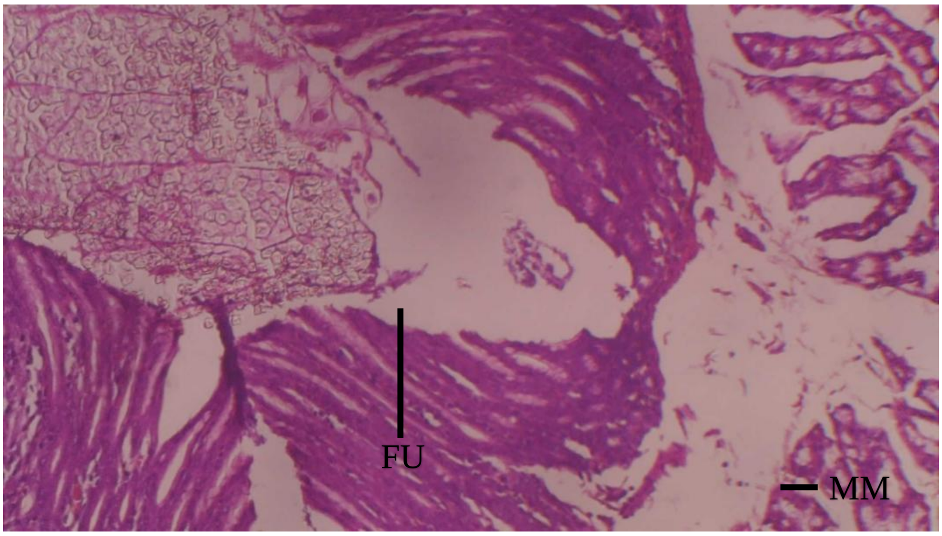

Plate 4. Rat duodenum given Alcohol only show: funnel-shaped ulcer (FU), muscularis mucosa (MM) : H&E 100 X

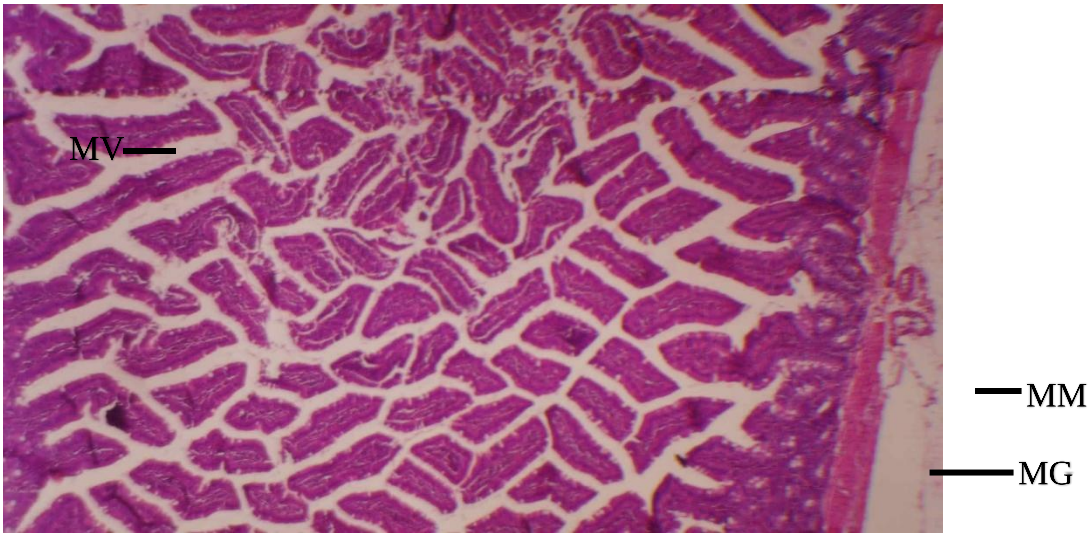

Plate 5. Rat duodenum given Alcohol + melatonin show: normal architecture: mucosal villi (MV), mucosal glands (MG), muscularis mucosa (MM): H&E 40 X

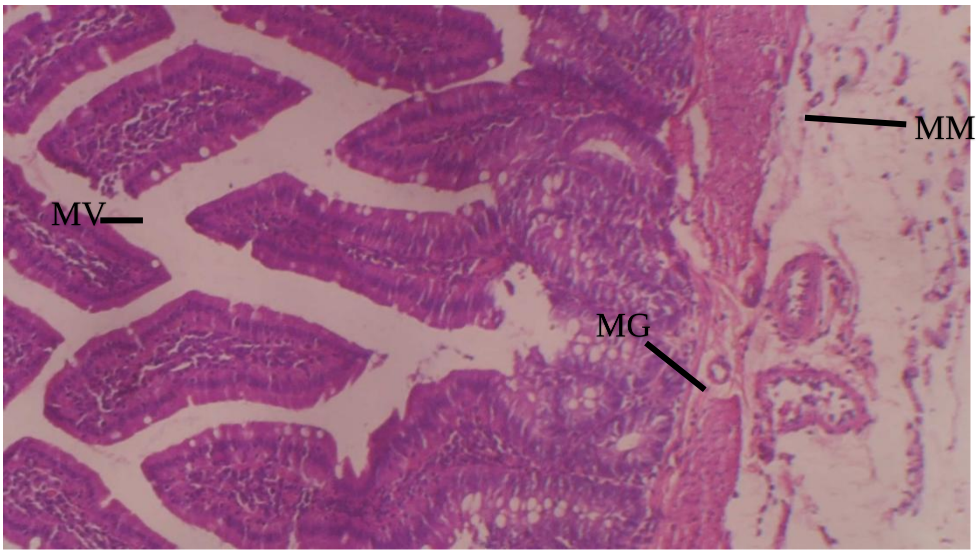

Plate 6. Rat duodenum given Alcohol + melatonin show: normal architecture: mucosal villi (MV), mucosal glands (MG), muscularis mucosa (MM) : H&E 100 X

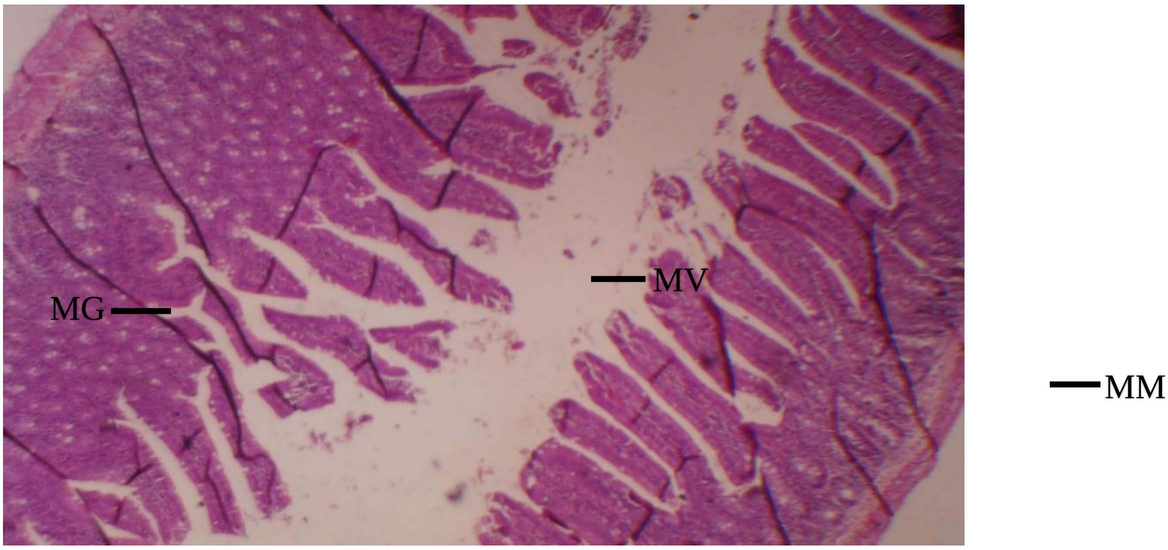

Plate 7. Rat duodenum given melatonin only show: normal mucosal villi (MV), mucosal glands (MG) and muscularis mucosa (MM): H&E 40 X

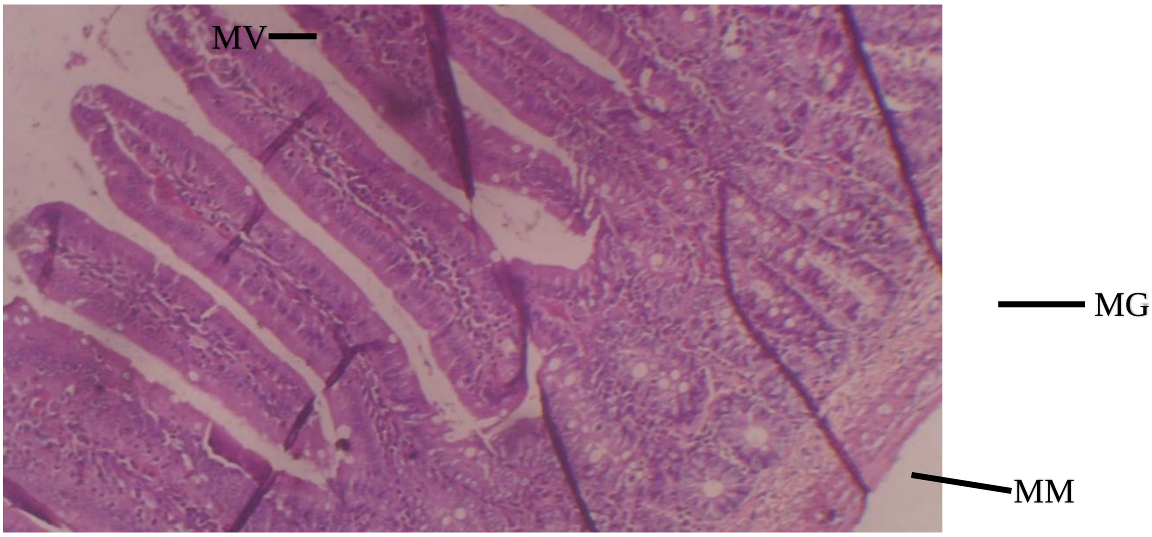

Plate 8. Rat duodenum given melatonin only show: normal mucosal villi (MV), mucosal glands (MG) and muscularis mucosa (MM) : H&E 100 X

CHAPTER FIVE

5.1 DISCUSSION

A decrease in body weight observed in the alcohol-treated group compared to the control group aligns with several earlier studies demonstrating the catabolic and appetite-suppressing effects of chronic alcohol consumption. According to Cederbaum (2012), ethanol metabolism increases energy expenditure and leads to oxidative stress, impairing nutrient absorption and resulting in reduced body weight gain. Similarly, Addolorato et al. (1998) reported that alcohol disrupts lipid and carbohydrate metabolism, contributing to loss of body mass due to altered hepatic function and poor energy utilization.

Conversely, the significant increase in body weight observed in the groups treated with melatonin only and melatonin combined with alcohol is consistent with findings from previous studies that highlight the metabolic regulatory role of melatonin. Prunet-Marcassus et al. (2003) and Ríos-Lugo et al. (2010) found that melatonin enhances insulin sensitivity, regulates lipid metabolism, and promotes energy balance by acting on adipose tissue and hypothalamic receptors. These effects contribute to improved metabolic efficiency and, in some cases, increased body weight under physiological or stress-induced conditions.

Moreover, melatonin’s antioxidant and anti-inflammatory properties may mitigate alcohol-induced oxidative damage in metabolic tissues, such as the liver and pancreas. Tomas-Zapico and Coto-Montes (2005) observed that melatonin protects against alcohol-related cellular injury, preserving mitochondrial function and improving nutrient utilization. This could explain the weight restoration or increase seen in the Melatonin + Alcohol group, as melatonin counteracts the catabolic effects of ethanol.

In line with these findings, Cardinali et al. (2012) suggested that melatonin supplementation helps normalize metabolic hormones such as leptin, ghrelin, and insulin, promoting appetite regulation and body weight maintenance. Therefore, the present observation that melatonin administration (alone or with alcohol) leads to a significant body weight increase supports existing literature that positions melatonin as a protective and metabolic regulatory agent against alcohol-induced metabolic disturbances.

The histological finding of a funnel-shaped ulceration in the duodenal mucosa of the alcohol-only group (Group D) agrees with earlier research showing that ethanol has a damaging effect on the gastrointestinal lining. Many studies have confirmed that alcohol can induce both duodenal and gastric ulcers through mechanisms involving cellular toxicity, oxidative stress, and inflammation of the mucosal barrier.

According to Szabo and Trier (1981), ethanol disrupts the integrity of mucosal epithelial cells by dissolving membrane lipids, which leads to cell death, bleeding, and ulcer formation. Likewise, Salim (1990) reported that alcohol reduces mucosal blood flow and increases acid back-diffusion, resulting in ischemic injury and deep tissue erosion. These findings are consistent with the funnel-shaped ulcer observed in the current study, suggesting that alcohol caused severe mucosal and submucosal destruction in the duodenum.

Keshavarzian et al. (1994) demonstrated that chronic alcohol intake promotes the generation of reactive oxygen species (ROS) and depletes antioxidant defenses such as glutathione and superoxide dismutase, making the mucosa more vulnerable to injury. This mechanism explains the degenerative histological changes observed in the alcohol-treated animals in this study.

Also Maity et al. (2003) described ethanol-induced mucosal damage as being characterized by inflammatory cell infiltration, vascular congestion, and epithelial loss, all of which were evident in the current findings (Plate 3). These similarities confirm that alcohol exerts direct toxic effects on the duodenal tissue, compromising its protective and regenerative abilities.

The protective and healing effects of melatonin (5 mg/kg) observed in this study against alcohol-induced duodenal injury are in line with previous research highlighting the compound's gastroprotective, antioxidant, and anti-inflammatory abilities. These findings suggest that melatonin helps reduce alcohol-related mucosal damage by neutralizing free radicals, strengthening mucosal defenses, and promoting tissue repair.

As reported by Bandyopadhyay et al. (2001), melatonin has strong antioxidant properties that allow it to neutralize reactive oxygen species (ROS) produced during alcohol metabolism, thereby preventing oxidative injury to the intestinal lining. Likewise, Reiter et al. (2000) described melatonin as a broad-spectrum protective agent that stabilizes cell membranes and increases the activity of key antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, all of which enhance the mucosa's resistance to ethanol-induced damage.

In agreement with the present study, Sener et al. (2002) demonstrated that administering 5 mg/kg of melatonin significantly reduced alcohol-induced gastric and duodenal lesions in rats by lowering lipid peroxidation and suppressing neutrophil infiltration. These effects were reflected in this study as restored mucosal structure and fewer ulcerative changes, showing both preventive and curative actions of melatonin.

Konturek et al. (1994) found that melatonin enhances prostaglandin synthesis, increases mucus and bicarbonate secretion, and improves mucosal blood flow, all of which support healing after alcohol-induced injury. These physiological actions may explain the recovery and tissue regeneration seen in the melatonin-treated group of the present research.

Büyükokuroğlu et al. (2002) also observed that melatonin significantly reduces oxidative gastric damage and promotes regeneration of epithelial cells, reinforcing its role as a natural protector of gastrointestinal tissues. The similarity between these findings and those of the current study further confirms that melatonin effectively counteracts alcohol-induced duodenal toxicity.

In conclusion, the protective and restorative effects of melatonin at 5 mg/kg observed in this study are supported by numerous experimental works. Together, they affirm melatonin's therapeutic potential in preventing and repairing alcohol-related gastrointestinal damage through its antioxidant, anti-inflammatory, and cytoprotective mechanisms.

5.2 CONCLUSION AND RECOMMENDATION

5.2.1 CONCLUSION

The results of this study show that alcohol intake has a strong toxic effect on the duodenum, which was reflected in both a decrease in body weight and visible histological damage, such as a funnel-shaped ulceration of the mucosal lining. These findings clearly indicate that ethanol negatively affects the structure and function of the gastrointestinal tract, compromising its overall integrity and metabolic balance.

5.2.2 RECOMMENDATION

From the present study, the following recommendations are made.

- Further long-term studies should be carried out to determine how different doses of melatonin affect alcohol-related damage to the gastrointestinal tract.

- Future research should also aim to understand how melatonin works at the molecular level, particularly its effects on oxidative stress, inflammation, and antioxidant enzyme activity.
- Since this research was conducted using animal models, it is important that clinical trials in humans be carried out to verify melatonin's effectiveness, safe dosage, and potential therapeutic benefits in protecting against alcohol-induced digestive disorders.
- Additionally, there should be increased efforts toward public health education to raise awareness about the harmful impact of excessive alcohol consumption on the digestive system and to encourage healthier lifestyle choices that can reduce alcohol-related health risks.

REFERENCES

Addolorato, G., Capristo, E., Greco, A. V., Stefanini, G. F., & Gasbarrini, G. (1998). Energy expenditure, substrate oxidation, and body composition in subjects with chronic alcoholism: New findings from indirect calorimetry. American Journal of Clinical Nutrition, 67(2), 594–599.

Andersen, L. P. H., Gögenur, I., Rosenberg, J., & Reiter, R. J. (2016). The safety of melatonin in humans. Clinical drug investigation, 36(3), 169-175.

Arendt, J. (2005). Melatonin: Characteristics, concerns, and prospects. Journal of Biological Rhythms, 20(4), 291–303.

Arendt, J. (2006). Melatonin and human rhythms. Chronobiology International, 23(1–2), 21–37.

Badria, F. A. (2002). Melatonin, serotonin, and tryptamine in some Egyptian food and medicinal plants. Journal of Medicinal Food, 5(3), 153–157.

Bancroft, J. D., & Gamble, M. (2008). Theory and Practice of Histological Techniques (6th ed.). Churchill Livingstone.

Bandyopadhyay, D., Biswas, K., Reiter, R. J., Banerjee, R. K., & Bandyopadhyay, U. (2001). Melatonin protects against stress-induced gastric lesions by scavenging free radicals. Journal of Pineal Research, 31(3), 214–222.

Barile, F. A. (2019). Alcohols and Aldehydes. In *Barile's Clinical Toxicology* (pp. 361-379). CRC Press.

Bhattacharyya, A., Chattopadhyay, R., Mitra, S., and Crowe, S. E. (2014). Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiological reviews*, *94*(2), 329-354.

Bubenik, G. A. (2002). Gastrointestinal melatonin: Localization, function, and clinical relevance. Digestive Diseases and Sciences, 47(10), 2336–2348.

Büyükokuroğlu, M. E., Taysi, S., Polat, F., & Göçer, F. (2002). The role of melatonin in ethanol-induced gastric mucosal injury in rats. Journal of Pineal Research, 32(3), 231–236.

Caputo, F., Guarino, M., Casabianca, A., Lungaro, L., Costanzini, A., Caio, G., ... and De Giorgio, R. (2024). Effects of Ethanol on the Digestive System: A Narrative Review. *Journal of Translational Gastroenterology*, *2*(4), 186-192.

Cardinali, D. P., Hardeland, R., & Srinivasan, V. (2012). Melatonin and metabolic regulation: A review. Neuroendocrinology Letters, 33(3), 205–220.

Carey, F. A., & Giuliano, R. M. (2013). Organic Chemistry (9th ed.). McGraw-Hill Education.

Carrillo-Vico, A., Guerrero, J. M., Lardone, P. J., & Reiter, R. J. (2005). A review of the multiple actions of melatonin on the immune system. Endocrine, 27(2), 189–200.

Cederbaum, A. I. (2012). Alcohol metabolism. Clinical Liver Disease, 16(4), 667–685.

Cipolla-Neto, J., & Amaral, F. G. (2018). Melatonin as a hormone: New physiological and clinical insights. Endocrine Reviews, 39(6), 990–1028.

Claustrat, B., & Leston, J. (2015). Melatonin: Physiological effects in humans. Neurochirurgie, 61(2–3), 77–84.

Dubocovich, M. L., Delagrange, P., Krause, D. N., Sugden, D., Cardinali, D. P., & Olcese, J. (2010). International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein–coupled melatonin receptors. Pharmacological Reviews, 62(3), 343–380.

Hacışevki, A., and Baba, B. (2018). *An overview of melatonin as an antioxidant molecule: a biochemical approach*. IntechOpen.

Hardeland, R. (2011). Melatonin—A pleiotropic, orchestrating regulator molecule. Progress in Neurobiology, 93(3), 350–384.

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

Hardeland, R. (2019). Melatonin and the pathologies of weakened or dysregulated circadian oscillators. Journal of Pineal Research, 67(1), e12525.

Hattori, A., Migitaka, H., Iigo, M., Itoh, M., Yamamoto, K., Ohtani-Kaneko, R., & Hara, M. (1995). Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. Biochemistry and Molecular Biology International, 35(3), 627–634.

Iriti, M., Rossoni, M., & Faoro, F. (2006). Melatonin content in grape wine and its involvement in plant–animal–human health. Journal of Pineal Research, 41(3), 331–335.

Keshavarzian, A., Holmes, E. W., Patel, M., Iber, F., Fields, J. Z., & Pethkar, S. (1994). Leaky gut in alcoholic cirrhosis: A possible mechanism for alcohol-induced liver damage. American Journal of Gastroenterology, 89(10), 1901–1907.

Klein, D. C. (2007). Arylalkylamine N-acetyltransferase: “The Timezyme.” Journal of Biological Chemistry, 282(7), 4233–4237.

Kłysik, M., Kruk, J., Misiak, M., Łukaszewicz, M., & Czuba, Z. P. (2020). The role of melatonin in food and its influence on human health. Nutrients, 12(8), 2434.

Konturek, S. J., Konturek, P. C., Brzozowski, T., & Pawlik, W. W. (1994). Role of melatonin in upper gastrointestinal tract. Journal of Physiology and Pharmacology, 45(4), 535–552.

Korkmaz, A., Reiter, R. J., Topal, T., Manchester, L. C., Oter, S., & Tan, D. X. (2012). Melatonin: An established antioxidant worthy of use in clinical trials. Molecular Medicine, 18(1), 861–872.

Kulczykowska, E., Kalamarz-Kubiak, H., & Gozdowska, M. (2017). Circadian melatonin rhythm in fish: A review. Chronobiology International, 34(3), 435–449.

Kumar, J., Haldar, C., and Verma, R. (2021). Melatonin ameliorates LPS-induced testicular nitro-oxidative stress (iNOS/TNFα) and inflammation (NF-kB/COX-2) via modulation of SIRT-1. *Reproductive Sciences*, *28*(12), 3417-3430.

Kuo, C. H., Wu, L. L., Chen, H. P., Yu, J., and Wu, C. Y. (2024). Direct effects of alcohol on gut-epithelial barrier: Unraveling the disruption of physical and chemical barrier of the gut-epithelial barrier that compromises the host–microbiota interface upon alcohol exposure. *Journal of Gastroenterology and Hepatology*, *39*(7), 1247-1255.

Lerner, A. B., Case, J. D., & Heinzelman, R. V. (1959). Structure of melatonin. Journal of the American Chemical Society, 81(21), 6085.

Lerner, A. B., Case, J. D., Takahashi, Y., Lee, T. H., & Mori, W. (1958). Isolation of melatonin, the pineal gland factor that lightens melanocytes. Journal of the American Chemical Society, 80(10), 2587.

Maity, P., Biswas, K., Roy, S., Banerjee, R. K., & Bandyopadhyay, U. (2003). Smoking and the pathogenesis of gastroduodenal ulcer—Recent mechanistic update. Molecular and Cellular Biochemistry, 253(1–2), 329–338.

Maldonado, M. D., Moreno, H., & Calvo, J. R. (2009). Melatonin present in beer contributes to increase the levels of melatonin and antioxidant capacity of the human serum. Clinical Nutrition, 28(2), 188–191.

Mayo, J. C., Sainz, R. M., Tan, D. X., Antolín, I., Rodríguez, C., & Reiter, R. J. (2005). Melatonin and Parkinson's disease. Endocrine, 27(2), 169–178.

McCord, C. P., & Allen, F. P. (1917). Evidences associating pineal gland function with alterations in pigmentation. Journal of Experimental Zoology, 23(1), 207–224.

McDonnell, G., & Russell, A. D. (1999). Antiseptics and disinfectants: Activity, action, and resistance. Clinical Microbiology Reviews, 12(1), 147–179.

Mishalany, H. G., Al-Khawaja, S., & Elzein, E. (2018). Anatomical and clinical significance of the suspensory ligament of the duodenum (ligament of Treitz). Journal of Clinical Anatomy, 31(5), 682–690.

Morrison, R. T., & Boyd, R. N. (2011). Organic Chemistry (6th ed.). Prentice Hall.

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., ... and 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.**

O'Keefe, J. H., Bybee, K. A., & Lavie, C. J. (2007). Alcohol and cardiovascular health: The dose makes the poison…or the remedy. Mayo Clinic Proceedings, 82(1), 66–74.

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.

Pal, P. K., Bhattacharjee, B., Chattopadhyay, A., and Bandyopadhyay, D. (2019). Pleiotropic roles of melatonin against oxidative stress mediated tissue injury in the gastrointestinal tract: An overview. *Melatonin Research*, *2*(2), 158-184.

Pandi-Perumal, S. R., Srinivasan, V., Maestroni, G. J. M., Cardinali, D. P., Poeggeler, B., & Hardeland, R. (2006). Melatonin: Nature's most versatile biological signal? FEBS Journal, 273(13), 2813–2838.

Pérez-Llamas, F., Hernández-Ruiz, J., & Fernández-López, J. A. (2018). Melatonin in foods and its influence on human health. International Journal of Food Sciences and Nutrition, 69(3), 321–331.

Pigeon, W. R., Carr, M., Gorman, C., & Perlis, M. L. (2010). Effects of a tart cherry juice beverage on the sleep of older adults with insomnia: A pilot study. Journal of Medicinal Food, 13(3), 579–583.

Prunet-Marcassus, B., Desbazeille, M., Bros, A., Louche, K., Delagrange, P., Renard, P., ... & Casteilla, L. (2003). Melatonin reduces body weight gain in Sprague Dawley rats with diet-induced obesity. Endocrinology, 144(12), 5347–5352.

Reiter, R. J., Tan, D. X., Manchester, L. C., & Paredes, S. D. (2009). Melatonin and reproduction revisited. Biology of Reproduction, 81(3), 445–456.

Reiter, R. J., Tan, D. X., Manchester, L. C., & Paredes, S. D. (2014). Melatonin in edible plants (phytomelatonin): Identification, concentrations, bioavailability, and proposed functions. World Review of Nutrition and Dietetics, 103, 1–26.

Reiter, R. J., Tan, D. X., Osuna, C., & Gitto, E. (2000). Actions of melatonin in the reduction of oxidative stress: A review. Journal of Biomedical Science, 7(6), 444–458.

Ríos-Lugo, M. J., Cano, P., Jiménez-Ortega, V., Fernández-Mateos, P., Scacchi, P. A., Cardinali, D. P., & Esquifino, A. I. (2010). Melatonin effect on plasma adiponectin, leptin, insulin, glucose, triglycerides and cholesterol in normal and high-fat-fed rats. Journal of Pineal Research, 49(4), 342–348.

Saadat, S., Alam, A., & Reiter, R. J. (2021). Melatonin and human nutrition: A review. Nutrients, 13(10), 3476.

Salim, A. S. (1990). Removing oxygen-derived free radicals stimulates healing of ethanol-induced gastric ulcers in the rat. Digestive Diseases and Sciences, 35(10), 1229–1234.

Sener, G., Paskaloglu, K., Sehirli, A. O., Ercan, F., Gedik, N., & Alican, I. (2002). Melatonin treatment protects against ethanol-induced gastric mucosal damage in rats. Journal of Pineal Research, 33(4), 256–261.

Simko, F., & Paulis, L. (2007). Melatonin as a potential antihypertensive treatment. Journal of Pineal Research, 42(4), 319–322.

Skandalakis, J. E., Colborn, G. L., & Weidman, T. A. (2014). Skandalakis' Surgical Anatomy: The Embryologic and Anatomic Basis of Modern Surgery (2nd ed.). Springer.

Smith, D. (2024). The pineal gland and its neurosecretory product, melatonin. *Medical Research Archives*, *12*(1).

Solomons, T. W. G., & Fryhle, C. B. (2016). Organic Chemistry (12th ed.). Wiley.

Stadler, R. H., Vernin, G., & De Saint Laumer, J. Y. (2018). Melatonin content in eggs: Influence of hen diet and circadian rhythm. Food Chemistry, 267, 500–506.

Stürtz, M., Cerezo, A. B., Cantos-Villar, E., & Garcia-Parrilla, M. C. (2011). Determination of the melatonin content of different varieties of tomatoes (Solanum lycopersicum L.). Journal of Food Composition and Analysis, 24(6), 911–916.

Szabo, S., & Trier, J. S. (1981). Ethanol-induced damage to the gastric mucosa: Pathogenesis and prevention. Gastroenterology, 80(2), 431–438.

Tan, D. X., Manchester, L. C., Esteban-Zubero, E., Zhou, Z., & Reiter, R. J. (2014). Melatonin as a potent and inducible endogenous antioxidant: Synthesis and metabolism. Molecules, 20(1), 188–210.

Tan, D. X., Manchester, L. C., Reiter, R. J., Qi, W. B., Zhang, M., Weintraub, S. T., Cabrera, J., Sainz, R. M., & Mayo, J. C. (2002). Identification of highly efficient melatonin metabolites (M^+ products). Journal of Biological Chemistry, 277(26), 23979–23986.

Tan, D. X., Manchester, L. C., Terron, M. P., Flores, L. J., & Reiter, R. J. (2007). One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? Journal of Pineal Research, 42(1), 28–42.

Tan, D.-X., Hardeland, R., Manchester, L. C., Paredes, S. D., Korkmaz, A., & Reiter, R. J. (2013). The changing biological roles of melatonin during evolution: From an antioxidant to signals of darkness, sexual selection, and fitness. Biological Reviews, 85(3), 607–623.

Tedor, M. F. (2021). Alcohol consumption and cultural systems: Global similarities and differences. *The handbook of alcohol use*, 355-378.

Tomas-Zapico, C., & Coto-Montes, A. (2005). Melatonin as antioxidant under pathological processes. Recent Patents on Endocrine, Metabolic & Immune Drug Discovery, 2(1), 63–82.

Tortora, G. J., Funke, B. R., & Case, C. L. (2016). Microbiology: An Introduction (12th ed.). Pearson Education.

Trease, G. E., & Evans, W. C. (2009). Pharmacognosy (16th ed.). Saunders Elsevier.

Walker, G. M. (2011). Bioethanol: Science and Technology of Fuel Alcohol. Springer.