INVESTIGATING THE EFFECT OF GLYCINE ON CADMIUM-INDUCED GASTRIC DAMAGE IN ADULT WISTAR RATS

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A PROJECT SUMMITTED TO DEPARTMENT OF ANATOMY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

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DEGREE IN ANATOMY.

SUPERVISED BY; DR. EDOBOR OBAYUWANA

SEPTEMBER, 2023

CERTIFICATION

I, **ERAYETAN YUSUF OLAMIDE,** hereby wish to certify that this research work presented in this dissertation for the award of master of Science (B.Sc.) degree in Anatomy is the result and solely of an independent research done by me under the supervision of Dr Edobor O. Obayuwana and any assistance given was duly acknowledged. I also certify that this Research work has not been submitted anywhere else in part or in full for any other examination or institution. All literatures and other sources of information consulted, cited or used in this research have been duly acknowledged with references.

_____ _____

Dr. EDOBOR O. OBAYUWANA DATE

(SUPERVISOR)

_____ _____

DR S.O INNIH DATE

(HEAD OF DEPARTMENT)

_____ _____

EXTERNAL SUPERVISOR DATE

DEDICATION

This work is dedicated to God Almighty for keeping me alive throughout my academic journey and especially throughout the duration of this research.

ACKNOWLEDGEMENTS

All praise be to the Almighty God for making me capable of this achievement. My heartfelt thanks to my supervisor Dr. Edobor O. Obayuwana and family for their immense guidance, support and contributions in this work.

To My dear parent Mr Tahir Erayetan and Mrs Mosurat Erayetan, my brother Sikiru Erayetan and, my dear sister Taibat Erayetan thank you all for your support

I will especially like to thank Dr.Innih Silvanus Olu , MR Enoghase R. Joseph and MR Nwamgbada M. Samuel. Who made immense contribution at different aspect in this research. All my lecturers of Human Anatomy Department, I thank you for your constant support.

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Table of Contents

ABSTRACT

An Experimental Study in Adult Wistar Rats Metal poisoning and its impact on human health have increased due to industrialization and anthropogenic activities. This study aims to investigate the effect of glycine on cadmium-induced gastric damage in adult Wistar rats. Thirty rats were divided into six groups, including control, cadmium only, glycine only, and combinations of cadmium and glycine. Various biochemical markers were assessed, including oxidative stress indicators (SOD, MDA, CAT) and total protein. Histological analyses were performed on stomach tissues. Cadmium administration led to reduced body weight and increased malondialdehyde (MDA) levels, indicating oxidative damage. Superoxide dismutase (SOD) activity decreased, revealing compromised antioxidant defenses. However, catalase activity was largely unaffected by cadmium. Interestingly, glycine administration showed positive effects. It attenuated cadmium-induced MDA increase, maintained glutathione levels, and improved SOD activity. It also increased total protein levels. Histological observations demonstrated that cadmium induced inflammatory responses, muscle degeneration, and congestion in the stomach. Glycine treatment mitigated these effects, leading to near-normal tissue architecture. This study demonstrates that cadmium exposure can lead to oxidative stress and cellular damage, while glycine supplementation can exert a gastro-ameliorative effect by enhancing antioxidant defenses, maintaining glutathione levels, and mitigating histological alterations. These findings offer insight into the potential therapeutic benefits of glycine against cadmium-induced gastric damage. Glycine's availability and safety make it a promising avenue for further research and development of affordable gastro-ameliorative interventions.

CHAPTER ONE

1.0 INTRODUCTION

1.1. Background of Study

Metal poisoning and its detrimental effects on human health have a long history. Modern industrialization and anthropogenic activities like mining, smelting, and home as well as agricultural usage of metals and metal-containing compounds have contributed to the extraordinary rise in metal exposure (Bradl, 2005). Metals, which are renowned for their high density and widespread existence on earth, can build up and have a negative impact on the ecosystem and living things (Briffa *et al*., 2020). Cadmium, cobalt, lead, mercury, aluminium, manganese, silver, uranium, vanadium, and zinc are a few of these heavy metals. Many people around the world experience metal poisoning as a result of contaminated water, food, and the environment. The chemical characteristics of metals and their capacity to interact with biological systems are related to their biological activities; this happens when one or more electrons are lost to form metal cations with high affinity to the nucleophilic sites of necessary macromolecules. According to reports, the effects of metal toxicity include gastrointestinal, stomach, and immune dysfunction, skin lesions, birth defects, cancer, and nervous system disorders. Metals accumulate in body tissues/cells, are transported there, and compartmentalise there before binding to proteins and nucleic acids and damaging macromolecules and impairing cellular functions.

A single hydrogen atom makes up the side chain of the amino acid glycine (Taghavinejad, 2008). The chemical formula of this amino acid is NH2CH2COOH, making it the most basic stable amino acid (carbamic acid is unstable). One of the amino acids that is proteinogenic is glycine.

All codons beginning with GG—GGU, GGC, GGA, and GGG—encode it. Due to its compact shape, glycine is essential for the creation of alpha-helices in secondary protein structure. It is the most prevalent amino acid in collagen triple helices for the same reason. A Clostridium tetani infection, for example, might interfere with the release of the inhibitory neurotransmitter glycine, which can result in spastic paralysis from unchecked muscular contraction. It is the sole achiral amino acid that can produce protein. Due to interference with its release within the spinal cord (such as during a Clostridium tetani infection), which can result in spastic paralysis from unchecked muscle contraction, it can fit into hydrophilic or hydrophobic environments since its minimum side chain contains only one hydrogen atom.

It is the sole achiral amino acid that can produce protein. Due to its little side chain—which contains just one hydrogen atom—it can exist in either hydrophilic or hydrophobic environments (Taghavinejad, 2008).

1.2 Aim of Study

This study aims to investigate the effec of glycine on cadmium-induced gastric damage in adult Wistar rats

1.3 Specific Objectives of study

The specific objectives of this study are to determine the

1. Changes in the body weight of Wistar rats across all groups
2. Changes in the organ weight of Wistar rats across all groups
3. Changes in Oxidative stress markers (SOD, MDA, CAT) of cadmium and glycine on the stomach of adult Wistar rats across all groups.
4. Changes in the histology of the stomach of adult Wistar rats across all groups.

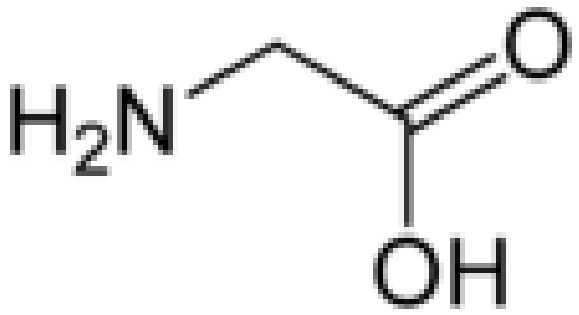
1.4 Significance of Study

This study contributes to expanding the range of glycine's therapeutic benefits. The results of this study will give insight into the hepato-protective properties of glycine, which may be an affordable, secure, and easily accessible treatment for hepatotoxicity with fewer side effects.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Glycine



Stucture of glycine

Merck Index (1989)

2.1.1 History and Etymology

French chemist Henri Braconnot discovered glycine in 1820 when he hydrolyzed gelatin by boiling it with sulfuric acid. (Plimmer, 1912) He initially called it "sugar of gelatin," but French chemist Jean-Baptiste Boussingault showed in 1838 that it contained nitrogen. (Boussingault, 1838) American scientist Eben Norton Horsford, then a student of the German chemist Justus. The name is derived from the Greek for "sweet tasting" (which is connected to the prefixes glyco- and gluco-, as in glucose and glycoprotein) (Oxford Dictionary, 2014). Auguste Cahours, a French scientist, discovered that glycine was an amine of acetic acid in 1858.) Cahours (1858).

2.1.2 Production

Although glycine can be extracted from hydrolyzed protein, chemical synthesis is the most practical method for producing it in commercial quantities. 2016) Okafor the Strecker amino acid synthesis, which is the primary synthetic method in the United States and Japan, and the amination of chloroacetic acid with ammonia, which results in glycine and ammonium chloride, are the two main procedures (USITC, 2014.). This method produces about 15,000 tonnes yearly. (Drauz, 2007) In the process of making EDTA, glycine is also produced as an impurity as a byproduct of processes involving the ammonia coproduct. (Hart, 2005)

2.1.3 Chemical Reaction

Most significant are its acid-base characteristics. Glycine is amphoteric in aqueous solution; below pH = 2.4, it transforms into the ammonium cation known as glycinium. As the pH rises above 9.6, it changes to glycinate. In order to create amino acid complexes, glycine serves as a bidentate ligand for several metal ions. Specifically, Cu(glycinate)2 is a typical complex. Cu(H2NCH2CO2)2 is an isomer that can be found in both cis and trans forms. Glycine transforms into amidocarboxylic acids such hippuric acid and acetylglycine when combined with acid chlorides (Ingersoll, A. and Babcock, 1932; Herbst and Shemin, 1939). Glycolic acid can be produced using nitrous acid (van Slyke determination). Trimethylglycine, a naturally occurring substance, is produced when the amine is quaternized with methyl iodide.

$$H_3N^+CH_2COO^- + 3\,CH_3I \rightarrow (CH_3)_3N^+CH_2COO^- + 3\,HI$$

Beginning with the synthesis of glycylglycine, glycine condenses with itself to produce peptides:

$$2\,H_3N^+CH_2COO^- \rightarrow H_3N + CH_2CONHCH_2COO^- + H_2O$$

Glycine or glycylglycine is pyrolyzed to produce 2,5-diketopiperazine, a cyclic diamide. With alcohols, it creates esters. They are frequently separated as their hydrochloride, for example, the hydrochloride of glycine methyl ester. In the absence of this, the free ester usually turns into diketopiperazine. The bifunctional molecule glycine interacts with a wide range of chemicals. These fall into two categories: N-centered reactions and carboxylate-center reactions.

2.1.4 Metabolism

2.1.4.1 Biosynthesis

Although glycine is not required for human nutrition because it can be biosynthesized in the body from serine, which is in turn derived from 3-phosphoglycerate, a supplement industry publication suggests that the metabolic capacity for glycine biosynthesis is insufficient to meet the demand for collagen synthesis. 2009 Melendez-Hevia The cofactor pyridoxal phosphate helps the enzyme serine hydroxymethyltransferase in most organisms catalyse this transformation: Nelson (2005). Glycine + N 5, N 10 -methylene tetrahydrofolate + H2O = serine + tetrahydrofolate Glycine synthase, also known as glycine cleavage enzyme, is the enzyme that catalyses glycine production in the liver of vertebrates. It is simple to reverse this conversion: Nelson (2005). CO_2 + NH^+_4 + N^5,N^{10} -methylene tetrahydrofolate + NADH + H^+ ⇌ Glycine + tetrahydrofolate + NAD^+. Glycine can be produced from serine, but it can also come from threonine, choline, or hydroxyproline thanks to the liver and stomach's inter-organ metabolism. (Wang, 2013)

2.1.4.2 Degradation

Three mechanisms are used to break down glycine. The glycine synthase pathway indicated above is not the primary pathway in animals or plants. The enzyme system at play in this

circumstance is commonly referred to as the glycine cleavage system: (Nelson and Michael, 2005) Metabolism Biosynthesis Degradation Glycine + tetrahydrofolate + $NAD^+ \rightleftharpoons CO_2 + NH^+_4$ + N^5,N^{10} -methylene tetrahydrofolate + NADH + H^+ Glycine undergoes two phases of degradation in the second pathway. With serine hydroxymethyl transferase, the initial step is the reversal of glycine production from serine. Serine dehydratase then converts serine to pyruvate. (Michael and Nelson, 2005). D-amino acid oxidase transforms glycine into glyoxylate in the third step of its breakdown. Hepatic lactate dehydrogenase then converts glyoxylate to oxalate in a process that is NAD +-dependent.(2005) Nelson and Michael Based on dose, glycine's half-life and rate of excretion from the body vary greatly. (Hahn, 1993). The half-life ranged between 0.5 and 4.0 hours in one research. (Hahn, 1993) Blood glycine levels drastically decrease within a minute following antibiotic infusions because glycine is very sensitive to antibiotics that target folate. Within a few minutes of administration, some antibiotics can deplete more than 90% of the amino acid glycine (Kwon *et al*., 2010).

2.1.5 Physiological Function

The main purpose of glycine is to serve as a protein precursor. A significant exception to this rule is collagen, which includes roughly 35% glycine because of its role in the production of collagen's helix structure in conjunction with hydroxyproline on a periodic basis. (Michael and Nelson, 2005; Szpak, 2011) Glycine is represented in the genetic code by all codons beginning with the letter GG, specifically GGU, GGC, GGA, and GGG.

2.1.5.1 As a biosynthetic intermediate

The enzyme ALA synthase creates -aminolevulinic acid, a crucial precursor of porphyrins, in higher eukaryotes from glycine and succinyl-CoA. The essential C2N subunit of all purines is provided by glycine. (Michael and Nelson, 2005; Szpak, 2011)

2.1.5.2 As a neurotransmitter

In the central nervous system, glycine functions as an inhibitory neurotransmitter, particularly in the retina, stomachstem, and spinal cord. Chloride enters the neuron through ionotropic receptors when glycine receptors are active, resulting in an inhibitory postsynaptic potential (IPSP). In contrast to bicuculline, strychnine is a potent antagonist at ionotropic glycine receptors. Along with glutamate, glycine serves as an essential co-agonist for NMDA receptors. Glycine plays an inhibitory effect in the spinal cord, however the excitatory (NMDA) glutamatergic receptors are where this behaviour is enabled. (2000) Liu and Zhang According to MSDS, 2005, glycine has an oral LD50 of 7930 mg/kg in rats and typically results in hyperexcitability-related death.

2.1.6 Uses

Glycine is commonly offered in two grades in the US: technical grade and United States Pharmacopoeia ("USP") grade. About 80 to 85 percent of glycine sales in the United States are USP grade. If, for instance, intravenous injections, purity greater than the USP standard is required, a more costly biological process as a biosynthetic precursor Utilised as a neurotransmitter, cisCu(glycinate)2 (H2O) (ITC,2008) Glycine of a pharmaceutical grade may be used. Technical grade glycine is sold at a lesser price for use in industrial purposes, such as as an agent in metal complexing and finishing, even though it does not exceed USP grade standards. Kasari (2004)

2.1.6.1 Animal and human foods

Except for infusions, glycine is not frequently added to foods for its nutritional value. Instead, glycine serves as a flavouring agent in food chemistry. It is subtly sweet and masks the saccharine aftertaste. Perhaps as a result of its complexation with metal ions, it also exhibits preservation qualities. Animal meals can be supplemented with metal glycinate complexes, such as copper(II) glycinate (Drauz and others, 2007).

2.1.6.2 Chemical feedstock

A number of different chemical compounds are synthesised using glycine as an intermediate. The herbicides glyphosate, (Stahl, 2016) iprodione, glyphosine, imiprothrin, and eglinazine are all made with it. (Drauz and others, 2007)

2.1.7 Laboratory research

Food sources of glycine (Raw, 2015)

Table 2.1

Food	g/100g
Snacks, pork skins	11.04
Sesame seeds flour (low fat)	3.43
Beverages, protein powder (soy-based)	2.37
Seeds, safflower seed meal, partially defatted	2.22
Meat, bison, beef and others (various parts)	1.5-2.0
Gelatin desserts	1.96
Seeds, pumpkin and squash seed kernels	1.82

Turkey, all classes, back, meat and skin	1.79
Chicken, broilers or fryers, meat and skin	1.74
Pork, ground, 96% lean / 4% fat, cooked, crumbles	1.71
Bacon and beef sticks	1.64
Peanuts	1.63
Crustaceans, spiny lobster	1.59
Spices, mustard seed, ground	1.59
Salami	1.55
Nuts, butternuts, dried	1.51
Fish, salmon, pink, canned, drained solids	1.42
Almonds	1.42
Fish, mackerel	0.93
Cereals ready-to-eat, granola, homemade	0.81
Leeks, (bulb and lower-leaf portion), freeze-dried	0.7
Cheese, parmesan (and others), grated	0.56
Soybeans, green, cooked, boiled, drained, without salt	0.51
Bread, protein (includes gluten)	0.47
Egg, whole, cooked, fried	0.47
Beans, white, mature seeds, cooked, boiled, with salt	0.38
Lentils, mature seeds, cooked, boiled, with salt	0.37

2.2 CADMIUM

Source:(Jacopo, 2018)

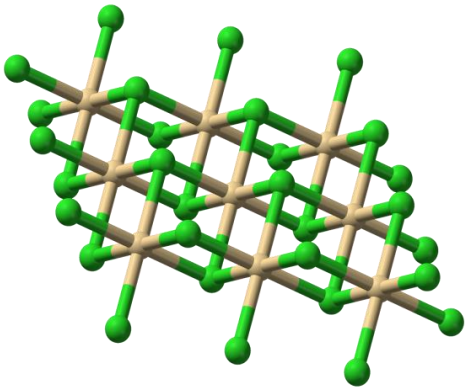
2.2.1 DEFINITION

Chemical element cadmium has the atomic number 48 and the letter Cd assigned to it. The two other stable metals in group 12—mercury and zinc—have chemical properties that are comparable to this delicate, silvery-white metal. Similar to zinc, it exhibits oxidation state +2 in the majority of its compounds, and it melts more slowly than the transition metals in groups 3 through 11. Since group 12's cadmium and its congeners lack partially filled d or f electron shells in either their

elemental or common oxidation states, they are frequently not regarded as transition metals. The Earth's crust contains between 0.1 and 0.5 parts per million (ppm) of cadmium on average. It was identified in 1817 as a zinc carbonate impurity by German scientists Stromeyer and Hermann at the same time.

Most zinc ores include trace amounts of cadmium, which is a byproduct of the zinc industry. Long employed as a corrosion-resistant coating for steel, cadmium compounds are also used to colour glass, stabilise plastic, and make red, orange, and yellow pigments. Because cadmium is hazardous, its use is typically declining (Morrow, 2000), and lithium-ion and nickel-metal hydride batteries have taken the place of nickel-cadmium batteries. Solar panels made of cadmium telluride are one of its few new applications. Although cadmium is not known to have any biological purpose in higher species, marine diatoms have been found to contain a cadmium-dependent carbonic anhydrase.

2.2.2 STRUCTURAL FORMULA



https://images.app.goo.gl/pXsbaZkGPhp3eqGm7

2.2.3 CHARACTERISTICS

Physical properties

Silvery-white, divalent cadmium is a soft, pliable, ductile metal. It creates complicated compounds but is similar to zinc in many ways. Cadmium is employed as a protective plate on other metals because it is resistant to corrosion, unlike the majority of other metals. Cadmium is not flammable and insoluble in water when it is in its bulk form, but it can burn and emit hazardous fumes when it is in powder form.

Chemical properties

Despite often having an oxidation state of +2, cadmium can also exist in the +1 state. Due to the fact that they lack partially filled d or f electron shells in their elemental or common oxidation states, cadmium and its congeners are not always regarded as transition metals. When cadmium burns in air, it produces brown amorphous cadmium oxide (CdO). The compound also exists in crystalline form, which is a dark red substance that changes colour when heated, much like zinc oxide. Cadmium is dissolved by hydrochloric, sulfuric, and nitric acids by producing cadmium chloride (CdCl2), cadmium sulphate (CdSO4), or cadmium nitrate (Cd(NO3)2). The Cd2 2+ cation, which is comparable to the Hg2 2+ cation in mercury (I) chloride, can be created by dissolving cadmium in a solution of cadmium chloride and aluminium chloride. This produces the oxidation state +1. (5) The structures of several cadmium complexes with nucleobases, amino acids, and vitamins have been identified. Cd + CdCl2 + 2 AlCl3 Cd2 (AlCl4)2.

2.2.4 USES OF CADMIUM

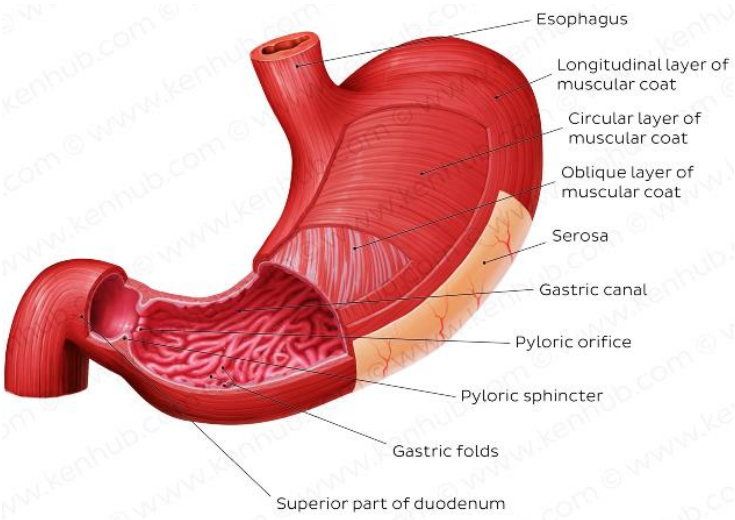
It can be used for the following:

1. Batteries (Krisgnamurthy, 2013)
2. Electroplating (Scoullous *et al*., 2001)
3. Nuclear fission (Scoullous *et al*., 2001)
4. Anticancer drugs (Abyae *et al*., 2019)
5. Televisions (Kwonspongsagoon, 2006).

2.3 Stomach

In the gastrointestinal tract of humans and many other animals, including numerous invertebrates, the stomach is a muscular, hollow organ. A crucial organ in the digestive system, the stomach has a dilated form. Following chewing, the gastric phase of digestion involves the stomach. It performs a chemical breakdown using hydrochloric acid and enzymes. The stomach is situated between the small intestine and the oesophagus in humans and many other animals. Gastric acid and digestive enzymes are secreted by the stomach to aid in food digestion. Chyme, or partially digested food, is moved from the stomach into the duodenum by the pyloric sphincter under the supervision of peristalsis, which then moves it through the remaining intestines (Nosek, 2016).

2.3.1 Structure



Standring, S. (2021).

The stomach is located in the human digestive system between the oesophagus and the duodenum, which is the first segment of the small intestine. The abdominal cavity's left upper quadrant is where it is located. The diaphragm is pressed up against the top of the stomach. The pancreas rests behind the stomach. The greater omentum, also known as the greater double fold of visceral peritoneum, hangs down from the greater curvature of the stomach. The lower esophageal sphincter (located in the cardiac region), at the intersection of the oesophagus and stomach, and the pyloric sphincter, at the intersection of the stomach and the duodenum, are the two sphincters that keep the contents of the stomach in check (Nosek, 2016). The stomach is surrounded by parasympathetic (stimulant) and sympathetic (inhibitor) plexuses (networks of blood vessels and nerves in the anterior gastric, posterior, superior and inferior, celiac, and myenteric), which regulate both the secretory activity of the stomach and the motor (motion) activity of its muscles. Because it is a distensible organ, it can generally expand to hold around one litre of food (Sherwood, 1997). A newborn human baby's stomach can only hold roughly 30

millilitres. The maximal stomach volume in humans is between 2 and 4 litres (Wenzel *et al*., 1998; Curtis and Sue 1994).

Sections

The human stomach is split into four divisions in classical anatomy, beginning with the cardia (Moore *et al*., 2014). The cardia is the passageway through which the contents of the oesophagus discharge into the stomach (Schwartz and Brunicardi, 2010). The fundus (Latin for 'bottom') is formed in the upper curved portion. The body is the stomach's major, core portion. The pylorus (from Greek 'gatekeeper') is the bottom part of the stomach that drains into the duodenum. The cardia is the region following the "z-line" of the gastroesophageal junction, when the epithelium transitions from stratified squamous to columnar. The lower esophageal sphincter is located near the cardia (Schwartz and Brunicardi, 2010). According to research, the cardia is not an anatomically unique part of the stomach, but rather a region of the esophageal lining that has been injured by reflux (Lenglinger *et al*., 2012).

Anatomical proximity

In mammals, the stomach bed refers to the structures on which the stomach rests (Habershon, 1909; Shearer and Tobin, 1981). These include the pancreatic tail, splenic artery, left stomach, left suprarenal gland, transverse colon and its mesocolon, diaphragmatic crus, and left colic flexure. Philip Polson of the Catholic University School of Medicine in Dublin coined the name around 1896. J Massey, a surgeon anatomist, brought this into question (Royal Academy of Medicine in Ireland, 1891).

2.3.2 Blood supply

The right gastric artery provides the inferior curvature of the human stomach and the left gastric artery supplies the superior curvature, as well as the cardiac area. The greater curvature is fed inferiorly by the right gastroepiploic artery and superiorly by the left gastroepiploic artery. The small gastric arteries, which emerge from the splenic artery, supply the fundus of the stomach as well as the upper portion of the greater curvature (Agur and Moore, 2007).

2.3.2 Histology

Wall

The human stomach walls, like the rest of the gastrointestinal tract, are made up of mucosa, submucosa, muscularis externa, subserosa, and serosa (Kenhub, 2021). The gastric mucosa, or inner lining of the stomach, is made up of an exterior layer of column-shaped cells, a lamina propria, and a thin layer of smooth muscle called the muscularis mucosa. The submucosa is a fibrous connective tissue layer beneath the mucosa (Kenhub, 2021). The Meissner's plexus is located in this layer, just beneath the oblique muscle layer (Welcome, 2018) The muscularis externa is a muscular layer located outside of the submucosa. It is made up of three layers of muscle fibres that are at right angles to each other. The inner oblique, middle circular, and outer longitudinal layers are these. The existence of the inner oblique layer distinguishes it from other regions of the gastrointestinal tract that lack it (Kenhub, 2021). Because the stomach has the thickest muscularis layer, consisting of three layers, greatest peristalsis occurs here. The inner oblique layer consists of: This layer is in charge of the motion that churns and physically breaks down the meal. It is the only layer of the three that is not found elsewhere in the digestive system. The antrum contains stronger skin cells in its walls and can contract more forcefully than

the fundus. The circular layer in the middle: The pylorus is bordered at this layer by a strong circular muscular wall that is generally tonically constricted, providing a functional (though not physically discrete) pyloric sphincter that controls the passage of chyme into the duodenum. This layer is concentric to the stomach's longitudinal axis. Auerbach's plexus (myenteric plexus) is located between the outer longitudinal and middle circular layers and innervates both (producing peristalsis and mixing). The outer longitudinal layer is in charge of pushing the bolus towards the stomach's pylorus by muscular shortening. A serosa, comprised of layers of connective tissue continuous with the peritoneum, lies to the exterior of the muscularis externa (Kenhub, 2021).

Glands

A number of these pits line the mucosa lining the stomach, which receive gastric juice released by between 2 and 7 gastric glands. Gastric juice is an acidic fluid that contains the digestion enzyme pepsin as well as hydrochloric acid. The glands include a variety of cells, and their function varies based on their location within the stomach. The fundic glands are located throughout the body and fundus of the stomach. Column-shaped cells release a protective coating of mucus and bicarbonate to line these glands. (2021, Kenhub).

Parietal cells secrete hydrochloric acid and intrinsic factor, main cells secrete pepsinogen (a precursor to pepsin- the very acidic environment changes the pepsinogen to pepsin), and neuroendocrine cells secrete serotonin (Dorland's 2012). Gallego-Huidobro and Pastor (1996) found that glands differ where the stomach enters the oesophagus and around the pylorus. Cardiac glands, which largely release mucus, are located between the confluence of the stomach and the oesophagus (Dorland's 2012). They are fewer in number than the other gastric glands and have a more normal antral mucosa microanatomy. Antral mucosa is made up of branched coiled

tubular glands bordered by secretory cells that resemble surface mucus cells. H&E stain, placed shallowly in the mucosa. There are two types: simple tubular with short ducts and compound racemose, which resembles the duodenal Brunner's glands. Pyloric glands are situated in the antrum of the pylorus near the pylorus. They exude mucus and gastrin, which is produced by their G cells (Dorland's 2012).

Gene and protein expression

In human cells, around 20,000 protein-coding genes are expressed, with nearly 70% of these genes expressed in the normal stomach (Uhlén *et al*., 2015). Approximately 150 of these genes are more specifically expressed in the stomach than in other organs, with just about 20 being extremely specific. The corresponding particular proteins expressed in the stomach are primarily engaged in producing a proper environment for managing food digestion and nutrient uptake. GKN1, which is expressed in the mucosa, pepsinogen PGC, and the lipase LIPF, which are expressed in chief cells, and gastric ATPase ATP4A and gastric intrinsic factor GIF, which are expressed in parietal cells (Gremel *et al*., 2015).

2.3.4 Embryology

During the initial stages of human embryogenesis, the ventral region of the embryo is adjacent to the yolk sac. As the embryo progresses into the third week of development and increases in size, it gradually encircles specific segments of the sac. These enveloped portions serve as the foundation for the eventual adult gastrointestinal tract (Schoenwolf *et al*., 2009). The yolk sac is encircled by a complex network of vitelline arteries and veins. As development continues, these arteries eventually consolidate into the three primary arteries responsible for supplying the developing gastrointestinal tract: the celiac artery, superior mesenteric artery, and inferior

mesenteric artery. The foregut, midgut, and hindgut are defined by the areas supplied by these arteries (Schoenwolf *et al*., 2009). The surrounding sac develops into the primitive intestine. Sections of this gut begin to differentiate into gastrointestinal tract organs, with the oesophagus and stomach forming from the foregut (Schoenwolf *et al*., 2009).

2.3.5 Function

Digestion

A bolus (a little circular lump of chewed up food) reaches the stomach via the oesophagus via the lower esophageal sphincter in the human digestive system. Proteases (protein-digesting enzymes like pepsin) and hydrochloric acid are released by the stomach, which kills or inhibits germs and gives an acidic pH of 2 for the proteases to operate. The stomach churns food through muscular contractions of the wall called peristalsis, lowering the volume of the bolus before looping around the fundus and the body of the stomach as the boluses are turned into chyme (partially digested food). Chyme moves slowly through the pyloric sphincter and into the duodenum of the small intestine, where nutritional extraction occurs. Pepsinogen is also present in gastric juice. This dormant type of enzyme, pepsin, is activated by hydrochloric acid. Proteins are broken down into polypeptides by pepsin (Richard and Marc (2007).

Absorption

Although absorption is mostly a function of the small intestine in the human digestive system, some absorption of some tiny molecules does occur in the stomach through its lining. This includes the following: If the body is dehydrated, drink water. Aspirin and other medications (Krehbiel and Matthew, 2015) Amino acids 10-20% of ethanol consumed (for example, from

alcoholic beverages) Caffeine is a stimulant (Debry, 1994). Water-soluble vitamin (most are absorbed in the small intestine) to a limited extent (McGuire and Beerman, 2017). The human stomach's parietal cells are in charge of creating intrinsic factor, which is required for vitamin B12 absorption. B12 is used in cellular metabolism and is required for red blood cell synthesis as well as nervous system function (McGuire and Beerman, 2017).

Control of secretion and motility

Through coordinated peristalsis and the opening of the pyloric sphincter, chyme from the stomach is slowly discharged into the duodenum. The autonomic nervous system and the digestive system's different digestive hormones both influence the movement and flow of substances into the stomach:

Gastrin	The hormone gastrin increases HCl production from parietal cells and pepsinogen secretion from main cells in the stomach. It also promotes increased gastric motility. Gastrin is secreted by G cells in the stomach in response to antrum distension and digestive products (particularly significant amounts of incompletely digested proteins). It is blocked by a pH less than 4 (high acid) and the hormone somatostatin.
Cholecystokinin	Cholecystokinin (CCK) has the largest effect on the gall bladder, inducing spasms, but it also reduces gastric emptying and increases the flow of alkaline pancreatic juice, which neutralises the chyme. CCK is produced by I-cells in the small intestine's mucosal epithelium.
Secretin	Secretin, which has the biggest effects on the pancreas, also reduces acid output in the stomach in a unique and unusual way. Secretin is produced by S-cells, which are found in both the duodenum and, to a lesser extent, the jejunum.
Gastric inhibiting peptide	GIP (gastric inhibitory peptide) inhibits both stomach acid secretion and motility. GIP is produced by K-cells, which are found in the duodenum and jejunum.
Enteroglucagon	Gastric acid and motility are both reduced with enteroglucagon.

These hormones, with the exception of gastrin, all work to inhibit stomach activity. This is in response to food ingredients that have not yet been absorbed in the liver and gallbladder. Only when the small intestine is empty does the stomach need to pump food into it. While the intestine is still processing meals, the stomach serves as food storage (McGuire and Beerman, 2017).

Chapter Three

MATERIALS AND METHOD

3.1 MATERIALS

Animals:30 Adult Wistar rats; Feed: Growers mash; Instruments: atman filter paper, funnel, conical flask, surgical blade, forceps,5ml syringe, laptop, weighing balance, microtome, slide tray, tissue embedding station, microscope, specimen bottles, cotton wool, orogastric tube, disposable gloves, measuring cylinder, pestle and mortar; Reagents:10% formal saline, chloroform, distilled water, eosin, haematoxylin, paraffin wax, xylene.

3.2 PURCHASE OF GLYCINE

Glycine and Cadmium was bought from Emmytex Biomedical stores at 47 New Lagos Road, opposite UBTH Benin City, Edo State.

3.4 METHOD

3.4.1 EXPERIMENTAL ANIMAL

Animal Care and management

Thirty adult Wistar rats were used as experimental animals in this study. Their weight ranged between 160 and 220g.The animals were purchased and maintained at the animal House in the Department of Anatomy, University of Benin, and we're transferred to their cages. Before transferring the rats into their cages, cages were cleaned and disinfected. The rats were left to acclimatize for a period of two weeks in their cages. They were fed with livestock's growers marsh manufactured by Top Feed limited, Sapele, Delta Stat, Nigeria throughout the

acclimatization period as much as they were allowed access to water. The cages were made of wood and wire gauze at the top to allow proper ventilation and the cages were cleaned daily and disinfected at intervals.

3.4.2 EXPERIMENTAL DESIGN

In this study, the animals were divided into six groups; A, B, C, D, E, and F with each group having five Wistar rats which were all weighed prior to the administration. The experimental period spanned for 28 days.

Thirty (30) adult Wistar rats weighing between 160g and 220g were separated into six (6) groups of randomized pattern with five (5) rats in each group.

TABLE 1.0: Experimental design

GROUPS	**DOSAGE**
Group A	Served as control and were fed with Animal feed and water for 14 days.
Group B	Received 10mg/kg of cadmium for 14 days.
Group C	Received 500mg/kg of glycine for 14 days.
Group D	Received 1000mg/kg of glycine for 14 days.
Group E	Received 10mg/kg of cadmium for 14 days and was treated with 500mg/kg of glycine for 14 days
Group F	Received 10mg/kg of cadmium for 14 days and was treated with 1000mg/kg of glycine for 14 days

Toxicity induction

Toxicity was induced by following a modification of binge – drinking model designed model designed by Carson and Pruett (Carson et.al., 1996)

3.4.3 METHOD OF SACRIFICE AND TISSUE COLLECTION

After 28 days, the animals were anesthetized with chloroform 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 blotted dry on a filter paper. The rats were weighed and their standard weights calculated using the following formula: Standard weight ={organ weight/(g)/body weight (g)} × 100. The liver tissues were then fixed for about 24 hours in 10% buffered formalin for routine haematoxylin and eosin histological processing and oxidative stress analysis.

STOMACH OXIDATIVE STRESS PARAMETERS

After harvesting the stomach, it was rinsed and weighed immediately using an electronic weighing balance calibrated in milligram and recorded to the nearest two decimal places. The harvested and weighed stomachs was washed twice in cold phosphate-buffered saline (PBS), homogenized using acid-washed sand and PBS in porcelain mortar and pestle. The homogenate was centrifuged at 10,000g for 15 minutes at 4°C. The supernatant was collected for the estimation of the various biochemical assays.

Estimation of Catalase (CAT) activity

This was determined by the method of Cohen *et al.*, (1970).

Principle

Catalase is present in nearly all animal, plant and bacteria cells. It acts to prevent the accumulation of noxious H_2O_2 which is converted to O_2 and H_2O.

Preparation of reagent

1. 0.01M $KMnO_4$ was prepared by dissolving 0.158g of $KMnO_4$ in 100ml of distilled water
2. Phosphate buffer (pH 7.4); 0.426 of $NaHPO_4$ NaH_2PO_4 was weighed and dissolved in 100ml of distilled water

- 6M H_2SO_4 : 32.3ml of conc. H_2SO_4 was added to 66.7ml of distilled water.
- 30Mm H_2O_2 solution: this was prepared by measuring 0.34ml of 30% of H_2O_2 in 1001ml of phosphate buffer.

Procedure

To a known volume of plasma, (0.5ml), 5.0ml of H_2O_2 was added. This was mixed by inversion and allowed to stand for 30 minutes. Reaction was stopped by adding 6M H_2SO_2

The absorbance was taken at 480nm within 30-60 seconds against distilled water.

Calculation

Activity = $\frac{\mathrm{OD} / \times \mathrm{min} \times Vt}{\mathrm{M} \times \mathrm{V} \times \mathrm{L} \times \mathrm{Y}}$

OD= absorbance

L= light path =1cm

V_t =total volume of reaction sample

M= molar extinction co-efficient of H_2O_2 (40/M/cm)

Estimation of Malondialdehyde (MDA) activity

Malondialdehyde was determined using the thiobarbituric acid assay (Buege and Aust, 1978).

Principle

Malondialdehydewhich is a product of lipid peroxidation reacts with thiobarbituric acid to give a red species.

Preparation of reagent

Stock TCA-TCB-HCL was prepared by mixing 15g of trichloroacetic acid, 0.375g of thiobarbituric acid and 0.25N hydrochloric acid. This solution was mildly heated to assist in the dissolution of the thiobarbituric acid.

Procedure

A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuging at 1000g for 10 minutes. The absorbance was determined at 535mn against a blank.

The concentration MDA was determined using the formula

MDA (unit/mg protein) = $\frac{A \times Vt \times 1000}{\mathrm{M} \times \mathrm{V} \times \mathrm{l} \times \mathrm{Y}}$

A = absorbance of sample test at 535nm

V_t= total volume of the reaction = 3ml

M = molar extinction co-efficient of product= $1.56 \times 10^5 m^{-1} cm^{-1}$

l = light path= 1cm

V= volume of tissue extract used = 1ml

Y = mg tissue in the volume of sample used

Estimation of Glutathione Peroxidase (GPx) activity

This was determined by the method of Ellman, (1959).

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown colourdisposition, read at 430nm.

Preparation of reagent

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100ml of distilled water.

Procedure

To an aliquot of plasma(0.2ml), 2.5ml of phosphate buffer, 2.5ml of H_2O_2, 1.5ml of distilled water and 2.5ml of pyrogallol was added. The reaction was allowed to stand for 30 minutes at room temperature. A deep brown color was formed which was read at 420nm.

Calculation

$$\text{activity} = \frac{\text{OD/Min} \times \text{VtDf}}{E \times V_s \times Y}$$

OD = Absorbance of test

V_t = Total volume of reaction of reaction mixture

Df = Dilution factor = l

E = Molar extinction coefficient (12/M/cm)

V_s= volume of sample

Y = mg of protein used

Superoxide dismutase (SOD) analysis

This was determined according to method of Misra and Fridovich (1972)

Principle

Adrenaline undergoes autoxidation rapidly to adrenochrome whose concentration can be determined at 420 nm with the aid of a spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions.

Superoxide dismutase inhibits auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420nm.

Preparation of reagents

Carbonate buffer (0.05 M) pH 10.2: this was prepared by dissolving 0.2014 g of Na_2CO_3. 0.2604g of $NaHCO_3$ and 0.0372g of EDTA in 100 ml of distilled water.

Hydrochloric acid (0.005 M): this was prepared by adding 0.044 concentrated HCl to 99.96 ml of distilled water.

Adrenaline solution (0.3 mM): this was prepared by dissolving 0.01098 g of Adrenaline in 100 ml of 0.005 M HCl solution.

Procedure

Plasma volume of 0.2 ml was mixed with 2.5 ml of carbonate buffer and 0.3 ml of adrenaline solution, 0.2 ml of distilled water was mixed with 2.5 m of carbonate buffer and 0.3 ml adrenaline as reference sample. These was mixed and absorbance read at 420 nm.

Calculation

$$\% \text{ inhibition} = \frac{(O.D_{test} - O.D_{ref}) \times 100}{O.D_{test}}$$

Enzyme activity can thus be calculated

$$\text{SOD activity (Unit/ mg protein)} = \frac{\% \text{ inhibition}}{50 \times Y}$$

Where Y = mg of protein in the volume of sample used.

3.5 HISTOLOGICAL TECHNIQUE

3.5.1 PARAFFIN EMBEDDING

The liver was excised and promptly transfered into 10% formal saline for fixation. Dehydration was carried out by passing the tissue through ascending grades of alcohol {70%, 90%, 95%, and 100% (absolute alcohol)} respectively for one hour. The tissue stayed in 70% alcohol for two hours, 90% alcohol for 18 hours (overnight) and 100% alcohol which was changed twice for two hours each. Clearing was carried out using xylene. The tissue was immersed in xylene for one hour so that alcohol will be completely removed. Infiltration of the tissue was carried out in an oven using molten paraffin wax at a temperature range of 30°C to 60°C for one hour. Three changes each at 15 minutes (twice) and 30 minutes (once) were carried out. Embedding was carried out using an embedding mould. The molten paraffin wax was poured into the embedding mould and the infiltrated tissues were placed in it. The orientation of the tissue was such that both longitudinal and transverse sections were cut. The molten paraffin wax was allowed to cool and form the tissue block. Prior to sectioning, the tissue blocks were trimmed and mounted on a wooden block holder. Sectioning was carried out a rotatory microtome. The tissue was clipped to the microtome and sectioned at the thickness of five microns. Sections came out as ribbon and we're placed in 20% alcohol for spreading of the tissue. The ribbons were cut and floated in water bath at a temperature of 30%. The sectioned tissue was placed in xylen for 5 minutes to remove paraffin wax from the tissue. Hydration was carried out by passing the tissues through descending grades of alcohol (100%,95%,100%, and 70%) for 5 minutes each.

3.5.2 STAINING

Haematoxylin and Eosin staining method

Staining was done using haematoxylin and eosin dyes. The tissues were stained in haematoxylin for 30 minutes and washed in water for 10 minutes. They were differentiated in 1% acid alcohol briefly and then washed in water. They were subsequently counterstained in Eosin for 2 to 3 minutes, and then rinsed in 90% alcohol

Dehydration was done with the sections passed through ascending grades of alcohol 70%, 90%, 95% for 30 seconds and in absolute alcohol for 2 seconds. The sections were immersed in xylene for 1 minutes. They were mounted in Discrete Plasticizer and Xylene (DPX), covered with coverslip using Canada balsam. Sections were viewed under a microscope.

3.6 PHOTOMICROGRAPHY

The sections of the liver were obtained and examined under leica DM750 research microscope with a digital camera (leica CC50) attached digital photomicrograph of the tissue were taken at X40, X100 and X400 objective magnifications.

3.7 STATISTICAL ANALYSIS

All collected disks were subjected to statistical analysis using Graph Pad Prism Version 9 and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data presented as mean ±SEM LSD post-hoc test was used. Values of $P<0.05$ were considered significant. The statistical values obtained were converted into graphical representations in form of bar charts.

CHAPTER FOUR

RESULTS

BIOCHEMICAL RESULT

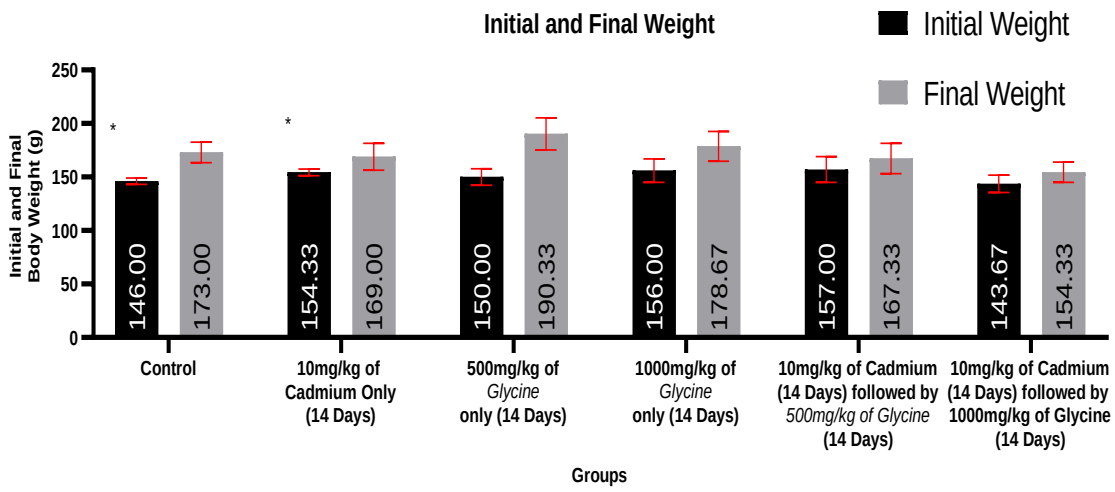


Chart 1 Showing Initial and Final Body Weight

*Represent statistically significant difference ($P<0.05$)

There was a statistically significant increase ($P<0.05$) in the final body weight (g) of animals in control group and 10mg/kg of Cadmium only for 14 days when compared with the initial body weight.

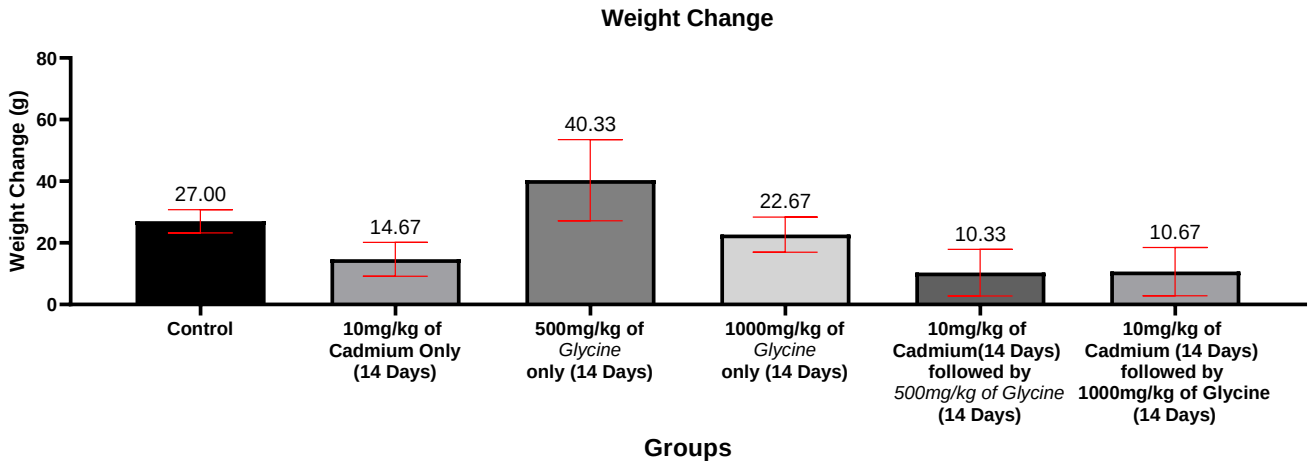


Chart 2 Showing Weight Change

There was no statistically significant difference ($P<0.05$) in Weight Change (g) for rats across the groups.

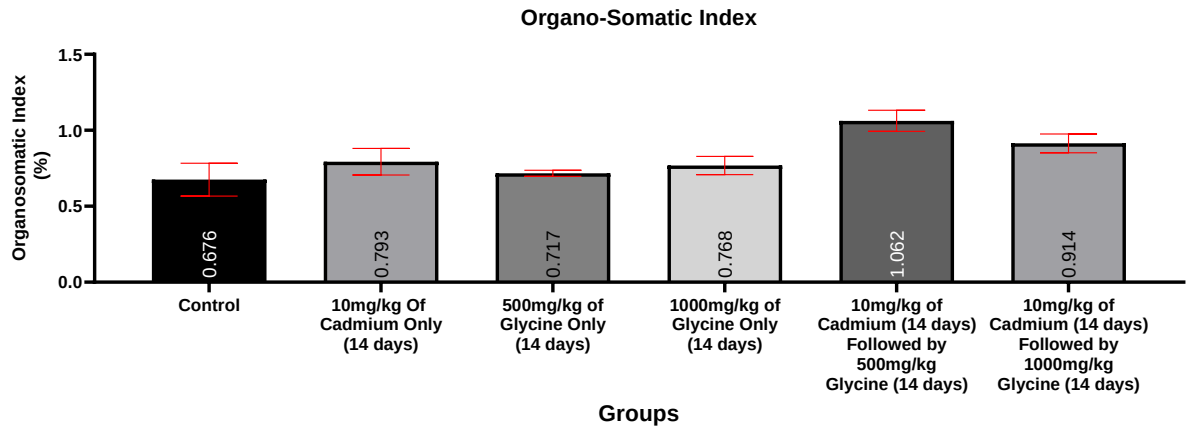


Chart 3 Showing Organo-Somatic Index

There was no statistically significant difference ($P<0.05$) in Organo-Somatic (g) for rats across the groups.

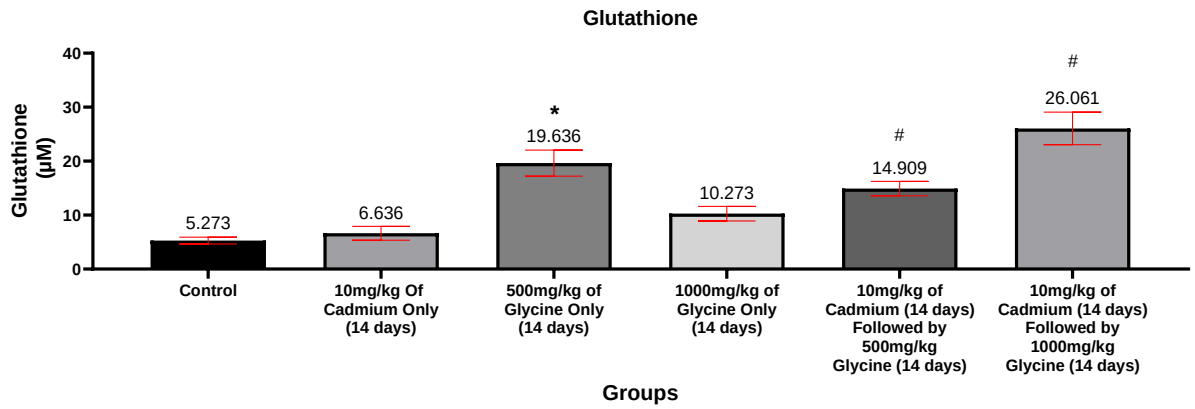


Chart 4 Showing Glutathione

*Represent Statistically significant difference ($p<0.05$) Compared with Control

#Represent Statistically significant difference ($p<0.05$) Compared with Cadmium only

There was a statistically significant increase ($p<0.05$) in Glutathione Levels (uM) for rats treated with 500mg/kg of Glycine only for 14 days when compared with control.

There was a statistically significant increase ($p<0.05$) in Glutathione Levels (uM) for rats treated with 10mg/kg of Cadmium for 14 days before administering 500mg/kg and 1000mg/kg of Glycine only for 14 days when compared with 10mg/kg of Cadmium for 14 days only.

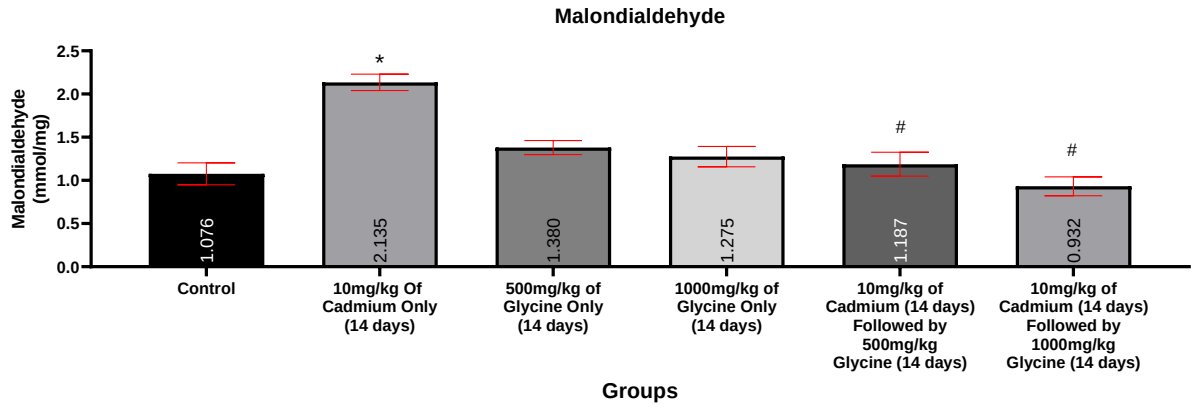


Chart 5 Showing Malondialdehyde

*Represent Statistically significant difference ($p<0.05$) Compared with Control

#Represent Statistically significant difference ($p<0.05$) Compared with Cadmium only

There was a statistically significant increase ($p<0.05$) in Malondialdehyde Levels (mmol/mg) for rats treated with 10mg/kg of Cadmium only for 14 days when compared with control.

There was a statistically significant decrease ($p<0.05$) in Malondialdehyde Levels (mmol/mg) for rats treated with 10mg/kg of Cadmium for 14 days before administering 500mg/kg and 1000mg/kg of Glycine only for 14 days when compared with 10mg/kg of Cadmium for 14 days only.

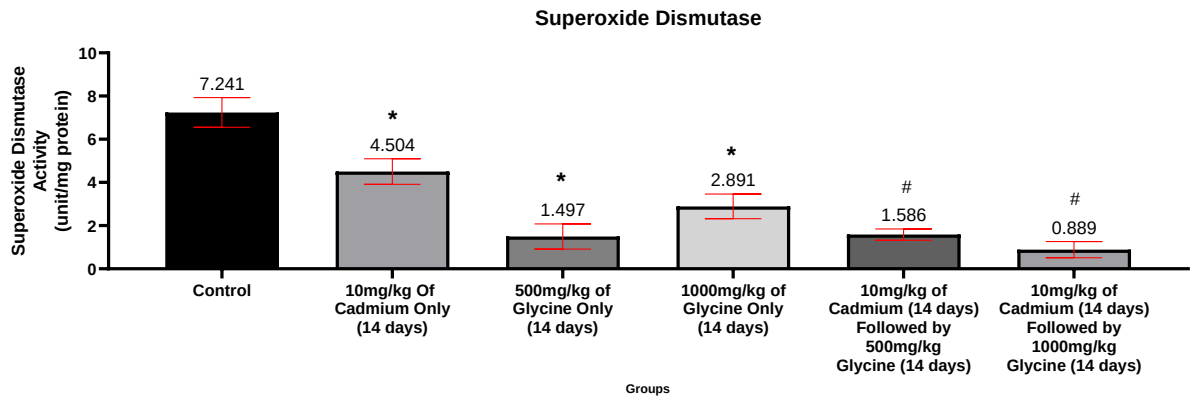


Chart 6 Showing Superoxide Dismutase Activities

*Represent Statistically significant difference ($p<0.05$) Compared with Control

#Represent Statistically significant difference ($p<0.05$) Compared with Cadmium only

There was a statistically significant decrease ($p<0.05$) in Superoxide Dismutase Activities (unit/mg) for rats treated with 10mg/kg of Cadmium only for 14 days; 500mg/kg and 1000mg/kg of Glycine only for 14 days when compared with control.

There was a statistically significant decrease ($p<0.05$) in Superoxide Dismutase Activities (unit/mg) for rats treated with 10mg/kg of Cadmium for 14 days before administering 500mg/kg and 1000mg/kg of Glycine only for 14 days when compared with 10mg/kg of Cadmium for 14 days only.

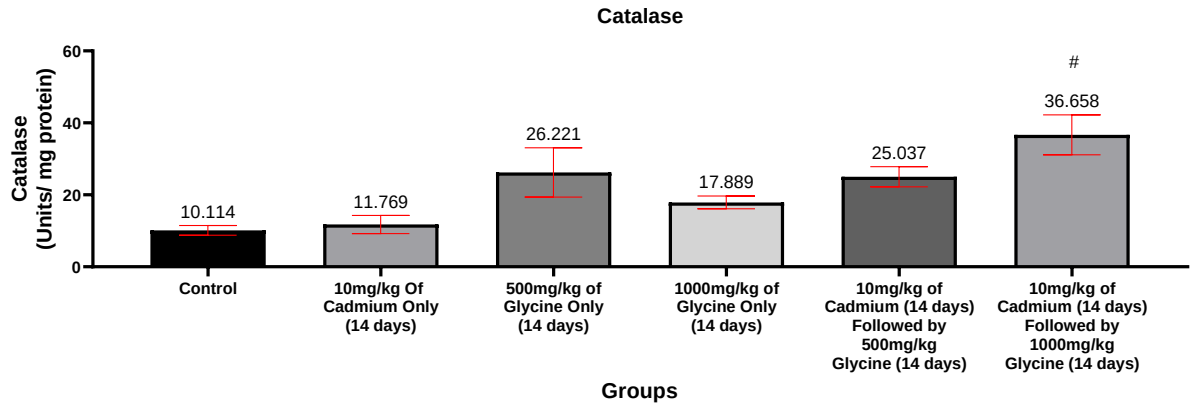


Chart 7 Showing Catalase Activities

#Represent Statistically significant difference ($p<0.05$) Compared with Cadmium only

There was a statistically significant increase ($p<0.05$) in Catalase Activities (unit/mg) for rats treated with 10mg/kg of Cadmium for 14 days before administering 1000mg/kg of Glycine only for 14 days when compared with 10mg/kg of Cadmium for 14 days only.

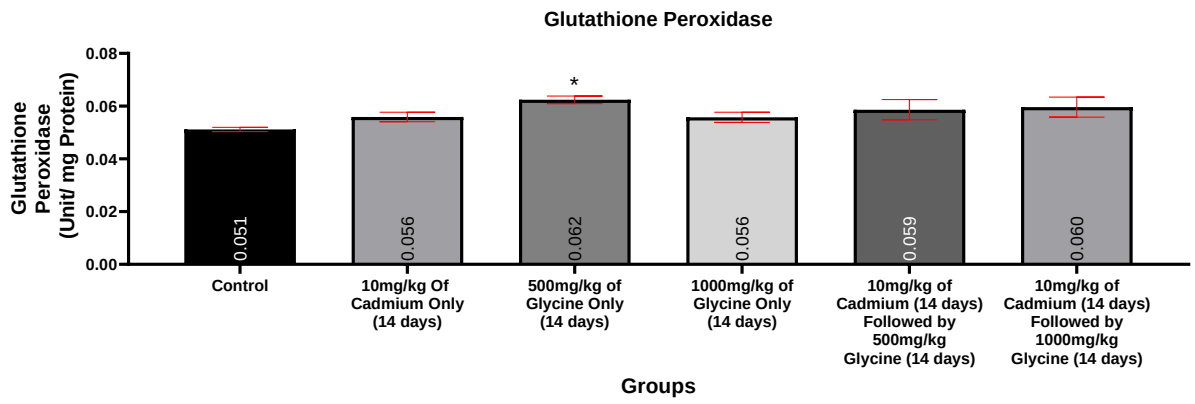


Chart 8 Showing Glutathione Peroxidase Activities.

*Represent Statistically significant difference ($p<0.05$) Compared with Control

There was a statistically significant increase ($p<0.05$) in Glutathione Peroxidase Activities (unit/mg) for rats treated with 500mg/kg of Glycine only for 14 days when compared with control.

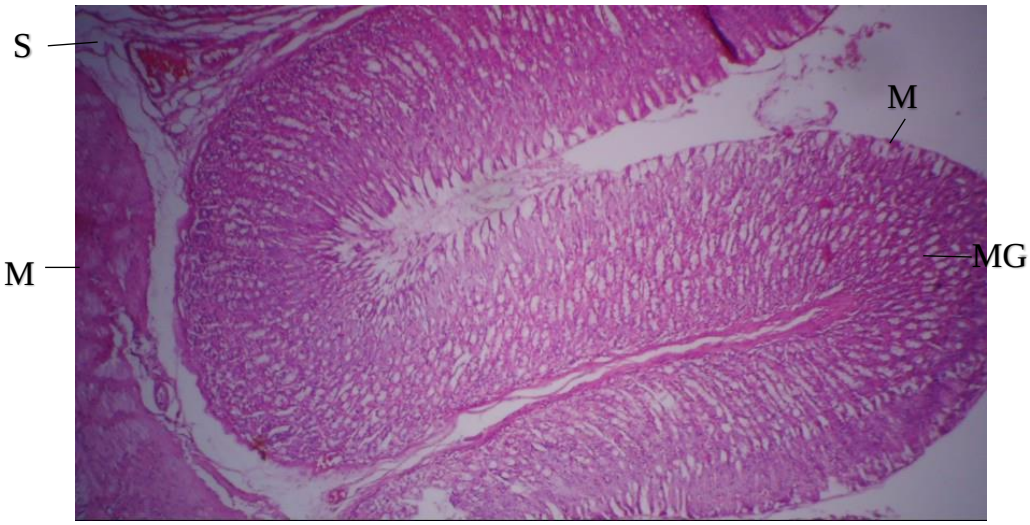


Plate 1. Rat stomach. Control. Composed of normal tissue architecture: mucosal epithelial lining indented by pits (ME), mucosal glands (MG), submucosa (SM), muscularis propria (MP): H&E x 40

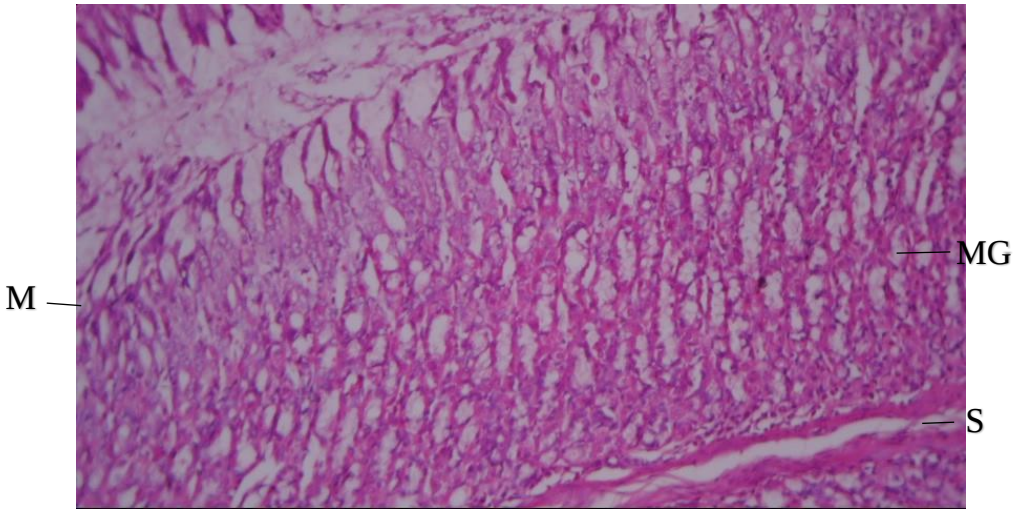


Plate 2. Rat stomach. Control. Composed of normal tissue architecture: mucosal epithelial lining indented by pits (ME), mucosal glands (MG), submucosa (SM), muscularis propria (MP): H&E x 100

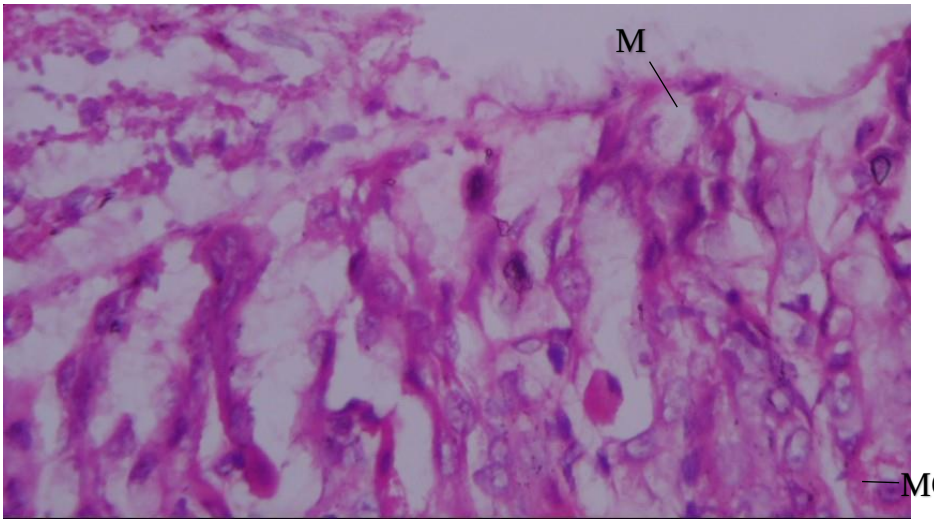


Plate 3. Rat stomach. Control. Composed of normal tissue architecture: mucosal epithelial lining indented by pits (ME), mucosal glands (MG), submucosa (SM), muscularis propria (MP) : H&E x 400

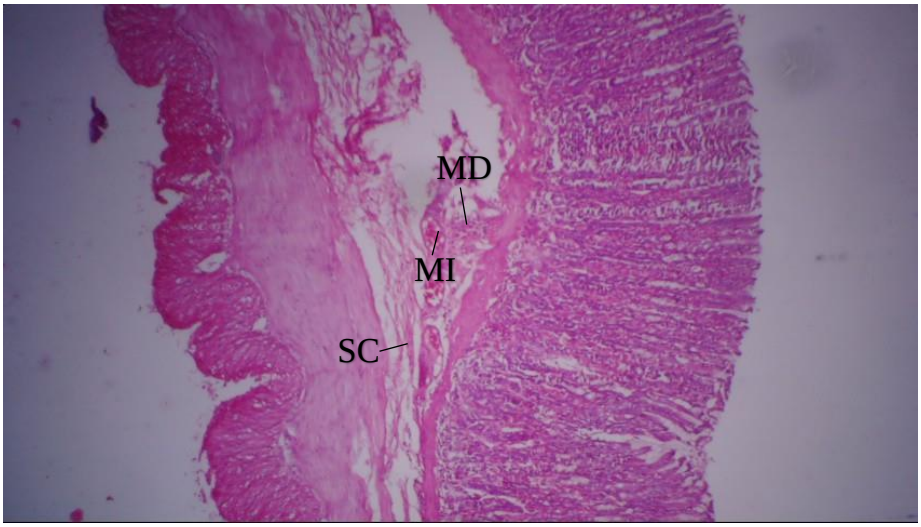


Plate 4. Rat stomach given 10mg/kg of Cadmium only showing: mucosal and submucosal infiltrates of inflammatory cells (MI), muscle degeneration (MD), submucosal congestion (SC): H&E X 40

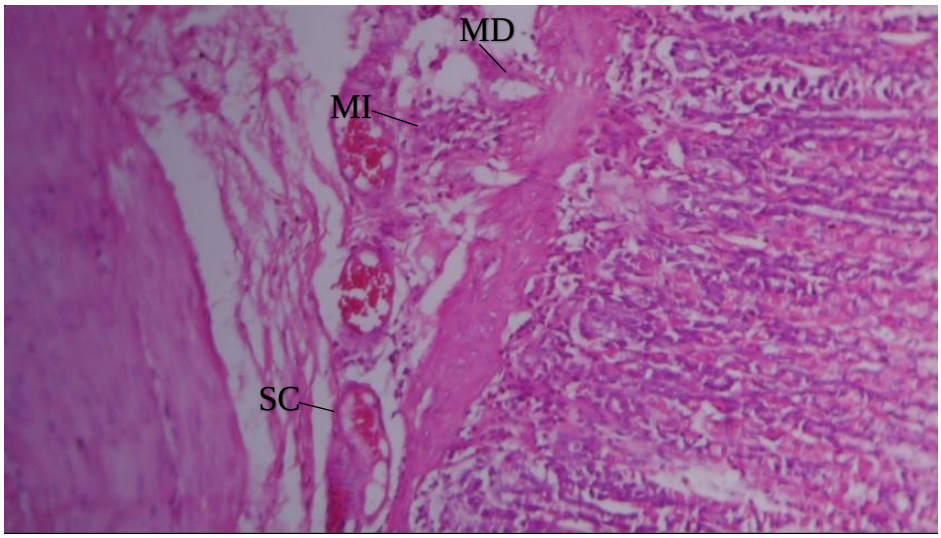


Plate 5. Rat stomach given Cadmium only showing: mucosal and submucosal infiltrates of inflammatory cells (MI), muscle degeneration (MD), submucosal congestion (SC) : H& E x 100

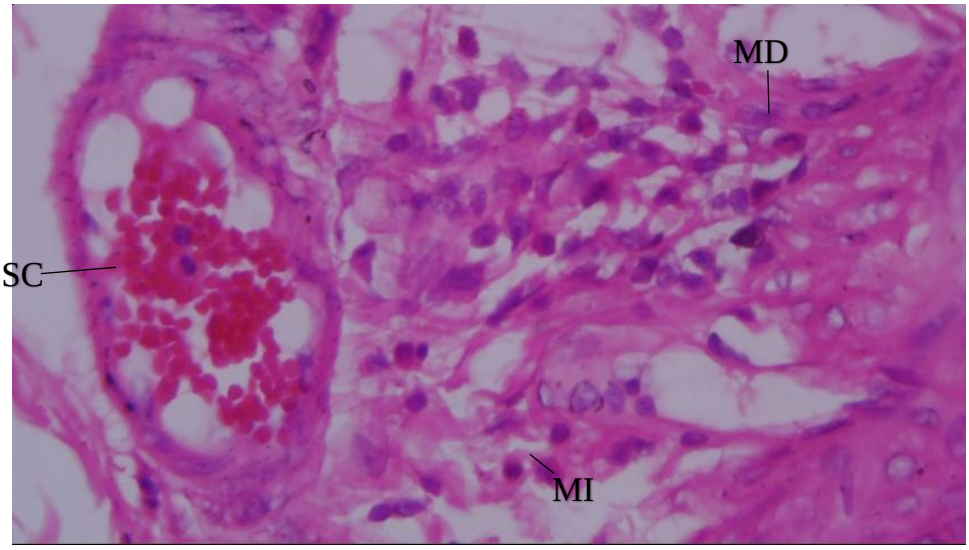


Plate 6. Rat stomach given Cadmium only showing: mucosal and submucosal infiltrates of inflammatory cells (ME), muscle degeneration (MD), submucosal congestion (SC) : H& E x 400

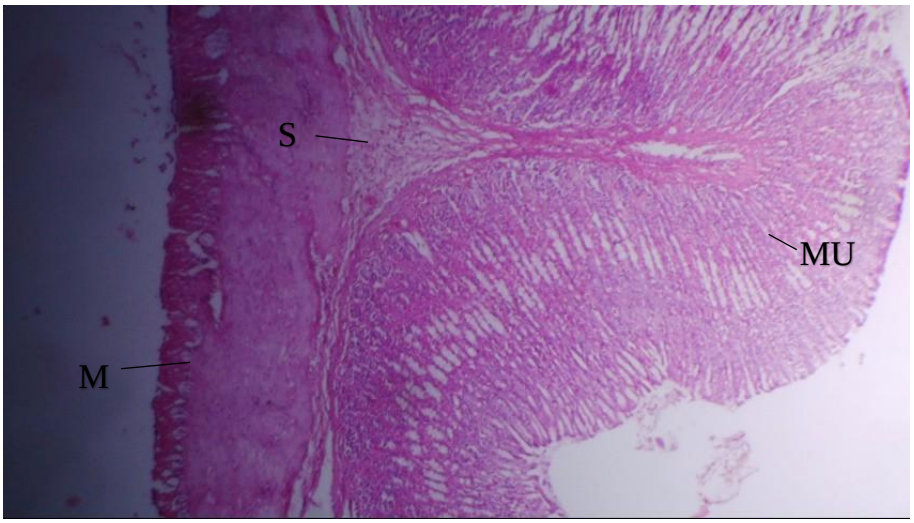


Plate 7. Rat stomach given 500mg/kg of Glycine only showing normal architecture: mucosa (MU), submucosa (SM), muscularis propria (MP): H&E x 40

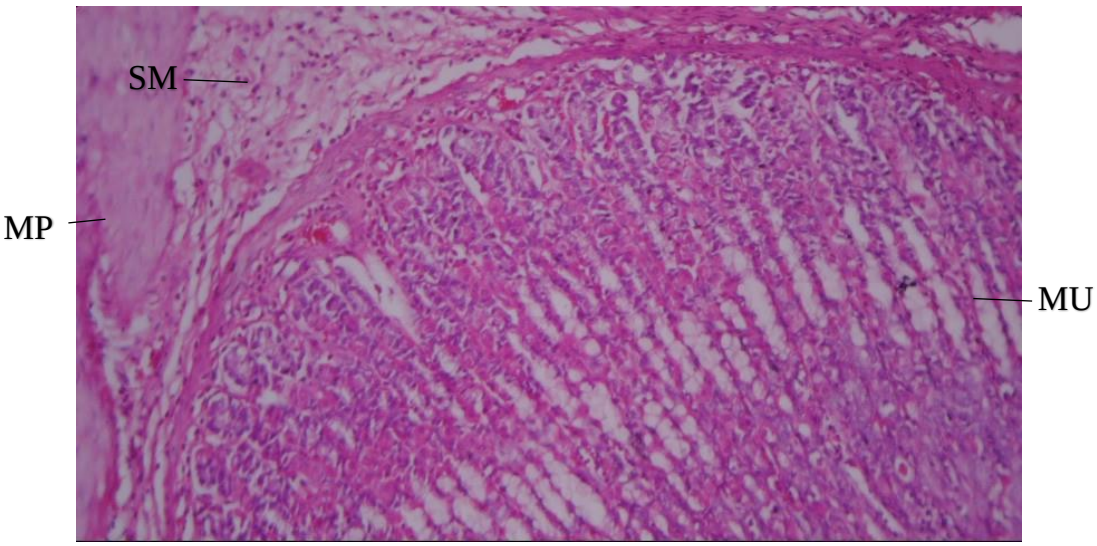


Plate 8. Rat stomach 500mg/kg of Glycine only showing normal architecture: mucosa (MU), submucosa (SM), muscularis propria (MP) : H&E x 100

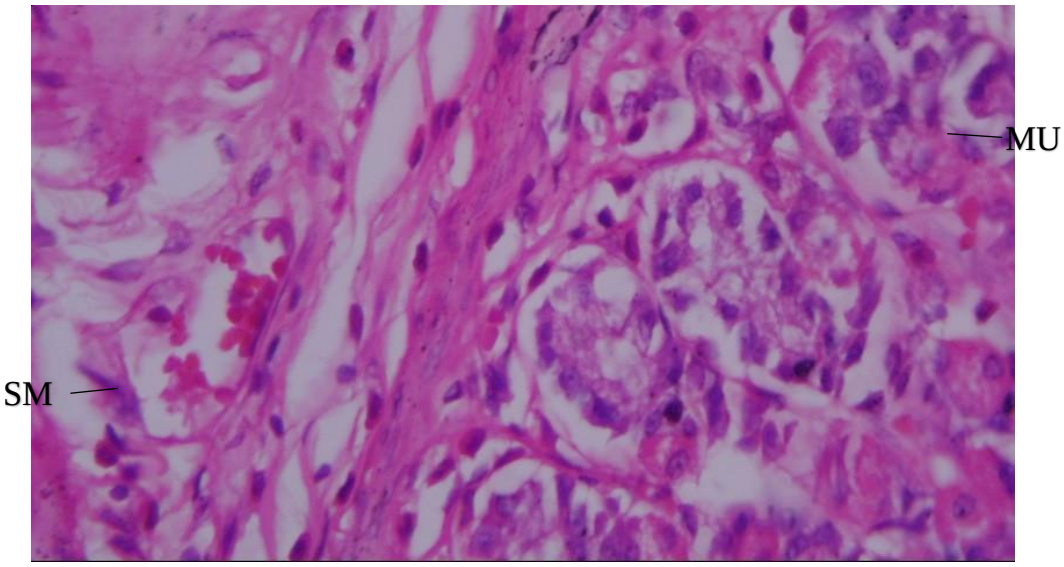


Plate 9. Rat stomach given 500mg/kg of Glycine only showing normal architecture: mucosa (MU), submucosa (SM), muscularis propria (MP) : H&E x 400

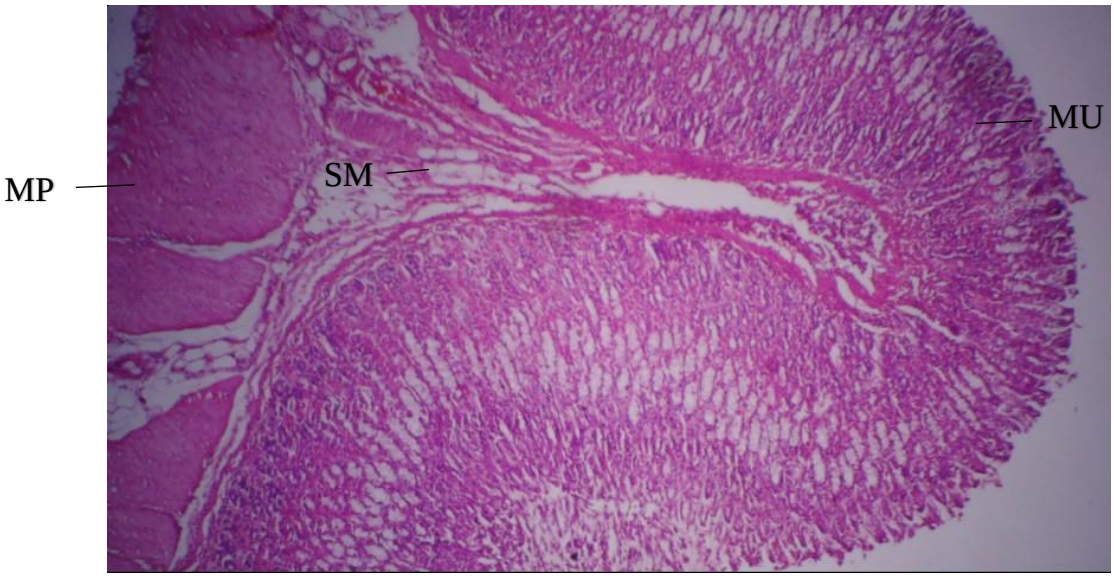


Plate 10. Rat stomach given 1000mg/kg of Glycine only showing normal architecture: mucosa (MU), submucosa (SM), muscularis propria (MP): H&E x 40

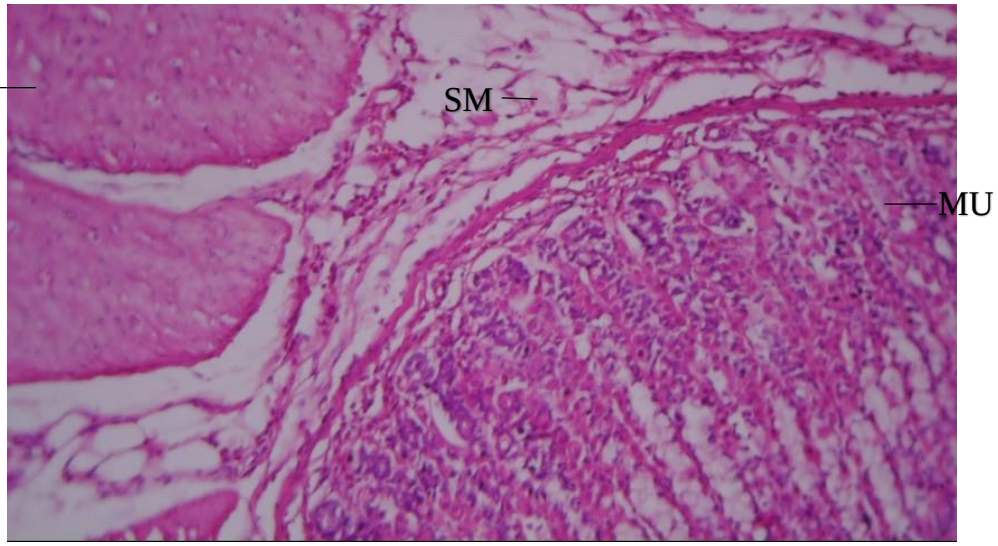


Plate 11. Rat stomach given 1000mg/kg of Glycine only showing normal architecture: mucosa (MU), submucosa (SM), muscularis propria (MP) : H&E x 100

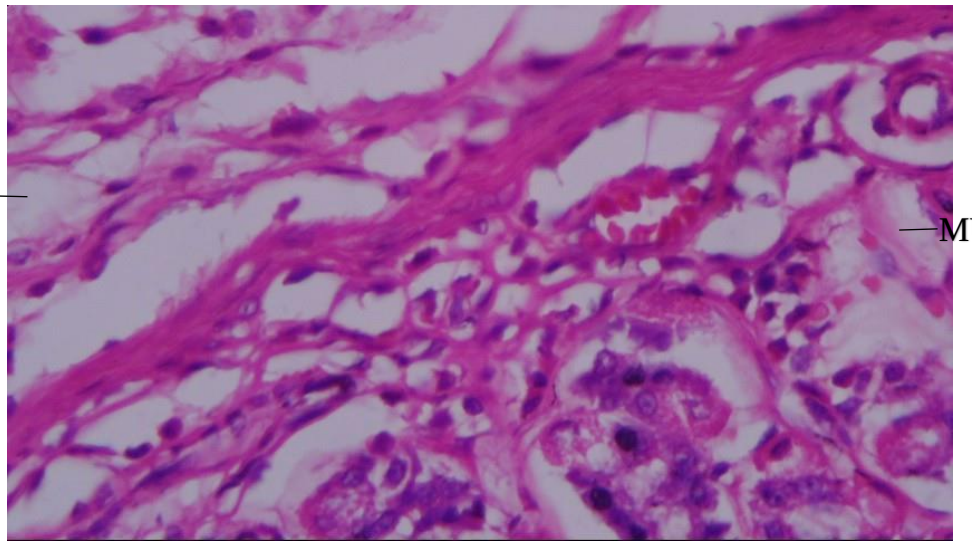


Plate 12. Rat stomach given 1000mg/kg of Glycine only showing normal architecture: mucosa (MU), submucosa (SM), muscularis propria (MP) : H&E x 400

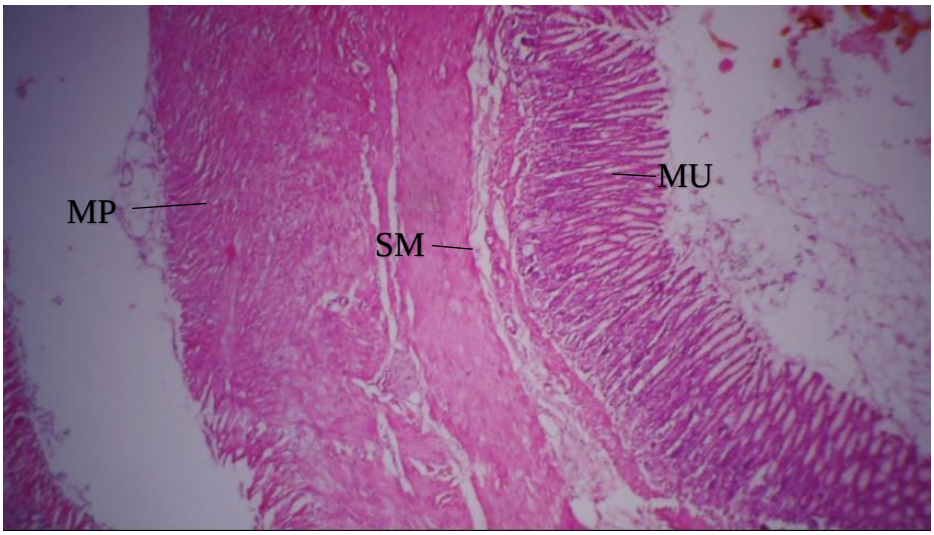


Plate 13. Rat stomach given Cadmium + 500mg/kg of Glycine showing normal architecture: mucosa (MU), submucosa (SM), muscularis propria (MP): H&E x 40

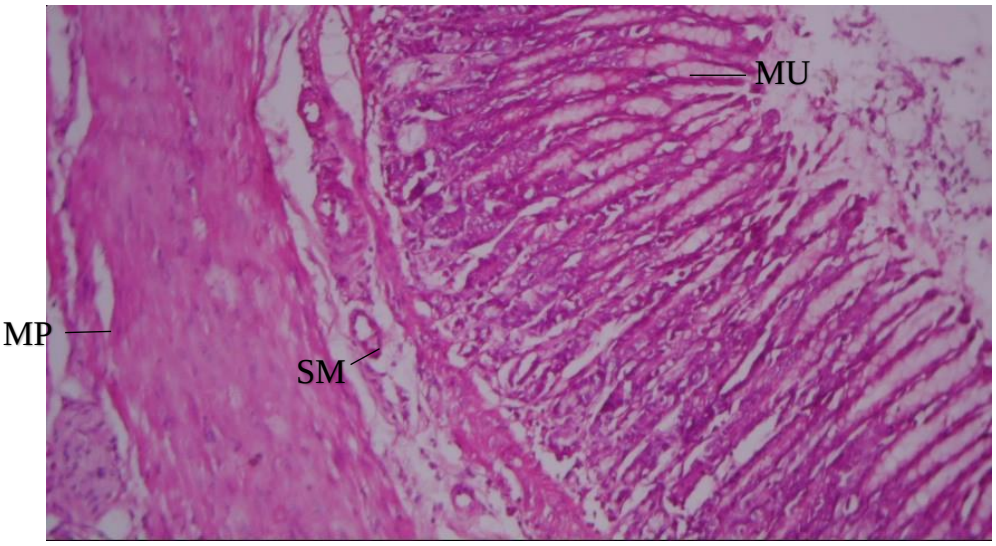


Plate 14. Rat stomach given Cadmium + 500mg/kg of Glycine showing normal architecture: mucosa (MU), submucosa (SM), muscularis propria (MP) : H&E x 100

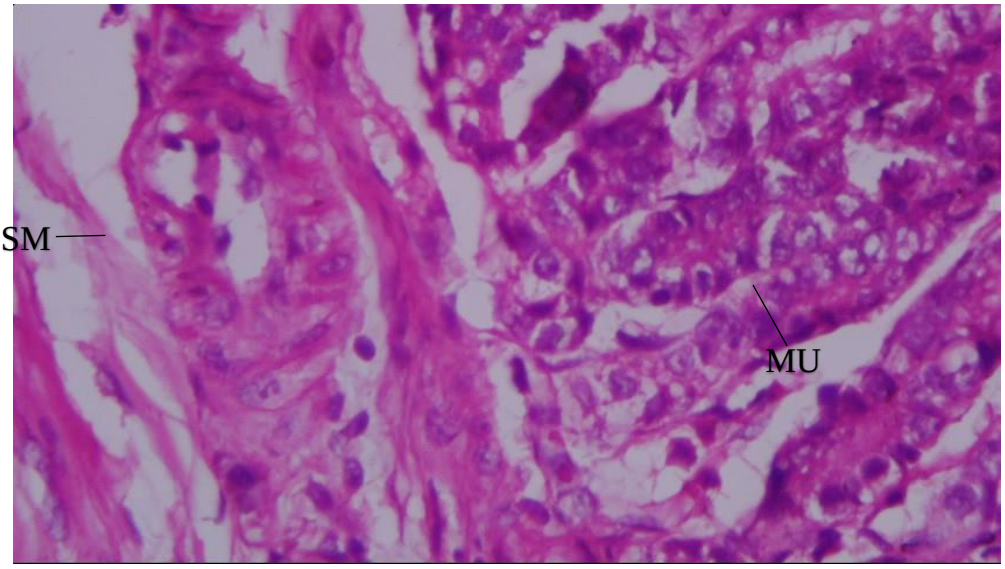


Plate 15. Rat stomach given Cadmium + 500mg/kg of Glycine showing normal architecture: mucosa (MU), submucosa (SM), muscularis propria (MP) : H&E x 400

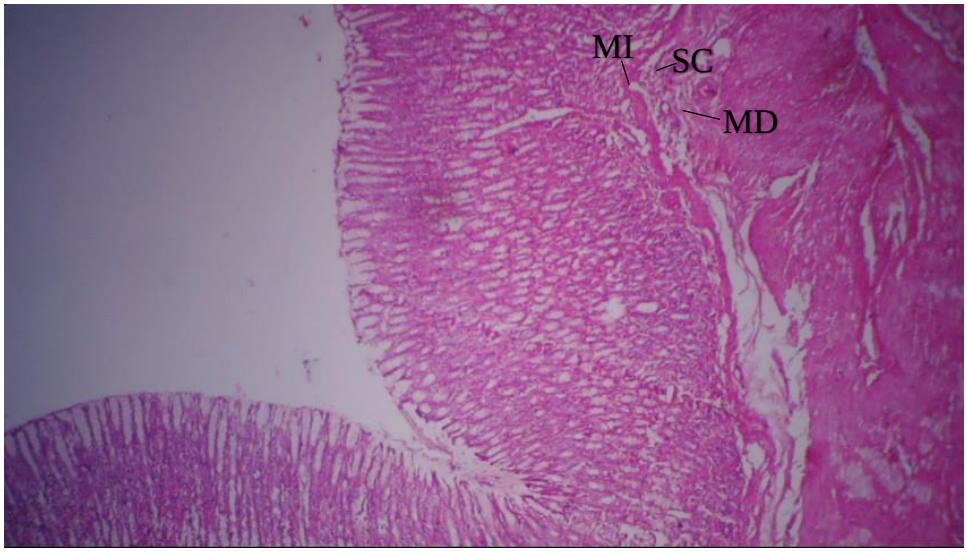


Plate 16. Rat stomach given Cadmium + 1000mg/kg of Glycine showing: mucosal and submucosal infiltrates of inflammatory cells (MI), submucosal congestion (SC), muscle degeneration (MD): H&E x 40

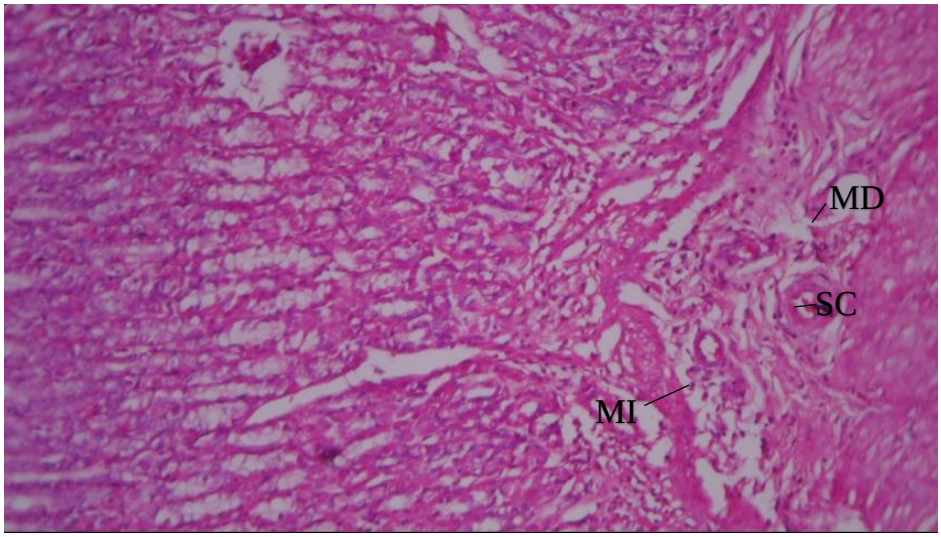


Plate 17. Rat stomach given Cadmium + 1000mg/kg of Glycine showing: mucosal and submucosal infiltrates of inflammatory cells (MI), submucosal congestion (SC), muscle degeneration (MD) : H&E x 100

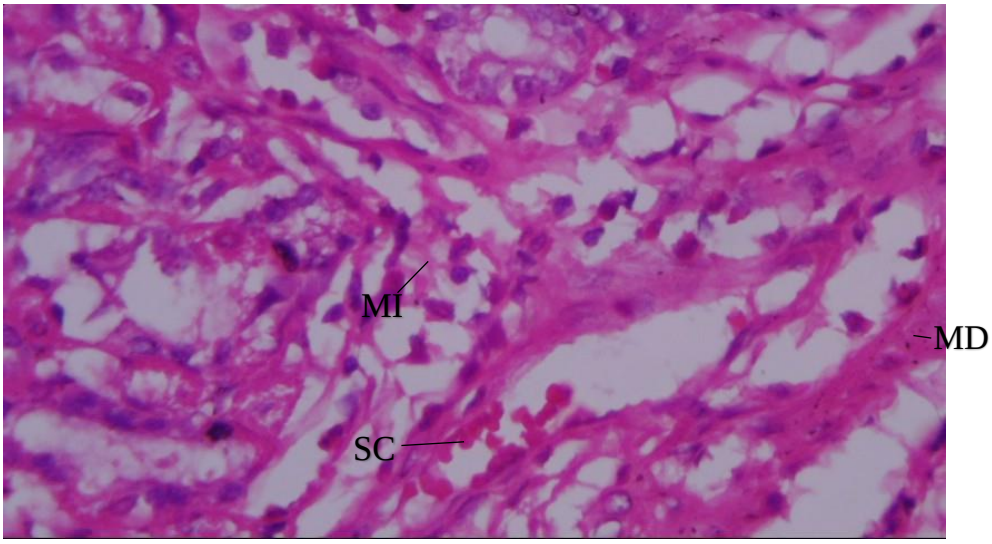


Plate 18. Rat stomach given Cadmium + 1000mg/kg of Glycine showing: mucosal and submucosal infiltrates of inflammatory cells (MI), submucosal congestion (SC), muscle degeneration (MD) : H&E x 400

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

DISCUSSION

An increase in the body weight was observed in all the groups as compared to their initial body weight. Although this increase was not significant, it was consistent across all groups. It has been argued that increase or decrease in either absolute or relative weight of an organ after administering a chemical or drug is an indication of the toxic effect of that chemical or drug (Orisakwe *et al*., 2003).

In comparing the mean body weight (Chart 2), it was observed that the body weight of the rats reduced after administration of 10mg/kg of cadmium when compared with control and when administered with 500mg/kg of glycine (an amino acid) the mean body weight increased when compared with control. Treatment of cadmium induced toxicity with 500mg/kg and 100mg/kg of glycine could not revert the weight loss caused by 10mg/kg of cadmium.

Cadmium, a toxic heavy metal, poses significant health risks to the body. Upon exposure, it can induce oxidative stress within cells, leading to damage of essential molecules (Muthukumar, 2010). Studies show that cadmium preferentially accumulates in the kidneys, with potential adverse effects on kidney function (Lane *et al*., 2000). Moreover, long-term exposure to cadmium is linked to increased risk of cancer, heart disease, and osteoporosis, underscoring its systemic impact on health (Luevano & Damodaran, 2014; Rahim *et al*., 2013; Tellez-Plaza *et al*., 2010; James & Meliker, 2013). Notably, even low levels of cadmium inhalation during childhood and adolescence can result in kidney uptake (Lane *et al*., 2000). Thus, understanding

the deleterious effects of cadmium is crucial for implementing measures to mitigate its potential harm to the body.

In this study, treatment with cadmium significantly increased the level of malondialdehyde as seen in chart 6, an increase in malondialdehyde levels serves as a signal that oxidative damage to cellular components, particularly lipids, is occurring (Halliwell *et al*., 2015). Glycine was able to reverse the increase in malondialdehyde levels caused by cadmium. Malondialdehyde (MDA) is a product of lipid peroxidation, which occurs when oxidative stress damages cell membranes' lipid components. While glycine might have antioxidant properties and contribute to reducing oxidative stress, reversing MDA levels that have already increased due to heavy metal exposure involves multiple intricate biochemical pathways.

Superoxide Dismutase was significantly reduced by 10mg/kg of cadmium after 14 days of administration as seen in chart 7; the reduction of SOD signifies compromised antioxidant defence mechanisms, leading to increased oxidative stress and potential negative consequences for cellular health and overall well-being. Glycine was also observed to cause a significant decrease in Superoxide Dismutase Levels; this can be attributed to various scenarios which can include:

Interference with Antioxidant Mechanisms: Glycine could potentially interfere with the cellular redox balance or antioxidant systems, indirectly affecting SOD activity. For example, excessive glycine might lead to imbalances that affect cellular metabolism, which could in turn impact the efficiency of antioxidant enzymes like SOD.

Complex Interactions: Glycine could interact with other molecules or pathways in a way that indirectly affects SOD. These interactions might lead to altered cellular signalling or metabolic processes, which could ultimately impact the regulation of SOD activity.

Catalase activity was not significantly affected by cadmium induction when compared with control, as seen in chart 8, 1000mg/kg of glycine was significantly increased after induction with 10mg/kg of cadmium. An increase in catalase activity is generally considered beneficial because it enhances the cell's antioxidant defences, reduces oxidative stress, and helps maintain cellular health. However, as with any biological process, the level of catalase activity should remain within a balanced range to avoid potential imbalances or unintended effects.

Glutathione Peroxidase was significantly increased when the experimental animals were administered with 500mg/kg of glycine only for 14 days, an increase in glutathione peroxidase activity is generally considered beneficial because it enhances the cell's ability to counteract oxidative stress, protect cellular components, and maintain overall cellular health.

Glutathione activities was significantly increased by glycine when compared with control and even when compared with rats administered with 10mg/kg of cadmium only (Chart 5). n increase in glutathione activity is generally considered beneficial because it enhances the cell's ability to combat oxidative stress, detoxify harmful compounds, repair damage, and maintain overall cellular health.

Glutathione activities increase is beneficial in the following context

Antioxidant Defense: Glutathione acts as a powerful antioxidant by neutralizing reactive oxygen species (ROS) and free radicals that can damage cellular components. An increase in

glutathione activity enhances the cell's ability to counteract oxidative stress (Lapenna *et al*., 1998).

Detoxification: Glutathione is involved in detoxifying harmful compounds, including environmental toxins and metabolic by-products. It helps in the neutralization and elimination of these substances from the body (Lay and Casida, 1976).

Repair of Oxidative Damage: Glutathione contributes to repairing oxidative damage to cellular structures such as proteins and DNA. Its role in maintaining cellular integrity is crucial for overall cellular health (Ceballos-Picot *et al*., 1996).

Immune Function: Glutathione supports immune function by protecting immune cells from oxidative damage. It also assists in regulating immune responses and maintaining proper immune cell function (Lu, 2013).

Cellular Redox Balance: Glutathione is a key player in maintaining the delicate balance between oxidized and reduced states (redox balance) within cells. This balance is essential for various cellular processes and signaling pathways (Semane *et al*., 2007).

Aging and Disease Prevention: Adequate glutathione levels are associated with healthy aging and reduced risk of chronic diseases. An increase in glutathione activity can contribute to these positive outcomes (Sian *et al*., 1994).

500mg/kg of glycine significantly increased the Total protein levels when compared with the control groups, Total protein levels can be influenced by a variety of physiological and pathological conditions. Therefore, whether an increase in total protein is considered "good" or not depends on the specific situation. These factors may include Normal Physiological Response,

Inflammatory Response, Nutritional Status, Underlying Conditions and Dehydration (Bosch *et al*., 1996).

Mucosal and submucosal infiltrates of inflammatory cells in the stomach refer to the presence of immune cells within the mucosal and deeper submucosal layers of the stomach lining. This phenomenon is often observed in response to inflammation, infections, autoimmune conditions, or other immune responses affecting the stomach (Hirasaki *et al*., 2005).

Treatment of rats with 10mg/kg of cadmium caused infiltration of inflammatory cells in the mucosa and submucosa layers of the stomach, including submucosa congestion. This effect was reversed after administration of 500mg/kg of glycine for 14 days as seen in chart 13 to 15.

Conclusion

From the present study, it is seen that 500mg/kg of glycine had an ameliorative effect on damaged caused to the stomach after cadmium toxicity. It can also be concluded that glycine is dose dependent as at higher dose of 1000mg/kg over similar time frame, glycine could not ameliorate the damage caused by cadmium toxicity to the stomach.

Recommendation

From this study, the following recommendation should be noted.

Glycine at 1000mg/kg could not ameliorate the effect caused by 10mg/kg of cadmium in the stomach; it is therefore recommended that further studies by carried out to ascertain the possible reason for this.

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