The Role of Glycine in Cadmium-Induced Liver Toxicity in Wistar Rats

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Abstract
Background/Objectives: The liver, a crucial organ responsible for regulating various physiological processes, is susceptible to harm from toxic substances like heavy metals. Cadmium, a toxic heavy metal, is naturally present in the environment. The surge in industrialization has resulted in increased cases of metal poisoning, causing a range of health problems. Glycine with anti-oxidants and anti-inflammatory properties has been demonstrated with some positive outcomes. This study aimed to investigate the role of glycine in liver injury induced by cadmium in Wistar rats. **Materials and Methods:** Thirty adult Wistar rats weighing between 150 and 170g were divided into six groups (Group A-F) of five rats per group and received 1 ml of distilled water (control), 10 mg/kg body weight of Cadmium, 500 mg/kg body weight of Glycine, 1000 mg/kg body weight of Glycine, 10 mg/kg body weight of Cadmium and 500 mg/kg body weight of Glycine, 10 mg/kg body weight of Cadmium and 1000 mg/kg body weight of Glycine respectively. At the end of the 14 and 28 days treatment, the rats were sacrificed using chloroform anaesthesia, blood and liver tissues were collected for analysis of serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) Albumin, total protein tests, and histology of the liver respectively. **Results and Conclusion:** There was significant decrease ($P<0.05$) in the body weights of rats compared to control. There was a significant decrease ($P<0.05$) in ALT and a significant increase ($P<0.05$) in AST in cadmium treated rats when compared to control. These effects were however reversed with glycine treatments. The total protein and albumin were also significantly decreased ($P<0.05$) in cadmium treated rats which were also reversed with glycine treatment. Histological findings showed infiltration of inflammatory cells, vascular congestion and cellular necrosis with cadmium treatment and these were ameliorated with 500 mg/kg of glycine treatment. Conclusively, glycine at 500 mg/kg dose possesses ameliorative and anti-inflammatory potentials against cadmium-induced liver injury.

Key words: Glycine, cadmium, anti-inflammatory, ameliorative, Wistar rats.

Introduction
The liver is a vital organ in the human body. It plays a major role in the regulation of many of the physiological processes in the body. These physiological processes include metabolism, secretory, storage and essential functions that are crucial for health and wellbeing (1). It is involved in detoxification of a variety of drugs and xenobiotic which makes it susceptible to the toxicity from these agents as the metabolic products of detoxification reaction can be destructive to the liver when in excess (2)

Heavy metals like cadmium are poisonous and naturally occurring in the earth crust. It is also release into the environment through various industrial activities like mining, smelting, battery manufacturing, and incineration of waste materials (3). Additionally, cadmium can contaminate

soil, water bodies, and agricultural products, leading to potential exposure in the general population (4). Metals accumulate in the body. Metal poisoning and its detrimental effects on human health has a long history. Modern industrialization and anthropogenic activities like mining, smelting, home and agricultural usage of metals and metal-containing compounds have contributed to the extraordinary rise in metal exposure (5). Metals, known for their high density and widespread existence on earth, can build up and negatively impact the ecosystem and living things (6). Cadmium, cobalt, lead, mercury, aluminum, manganese, silver, uranium, vanadium, and zinc are a few of these heavy metals. Many people around the world experience metal poisoning due to contaminated water, food, and the environment. The chemical features of heavy metals and their capacity to interact with biological systems are linked to their biological activities; which happens when one or more electrons are lost to form metal cations with high affinity to the nucleophilic sites of necessary macromolecules. They are often transported, and compartmentalized within bodily tissues and cells where they bind to proteins and nucleic acids, causing damage to macromolecules and impairing cellular processes (7).

Glycine is a non-essential amino acid in the body. It has a single hydrogen atom which makes up the side chain of the amino acid glycine (8). Although glycine can be extracted from hydrolyzed protein, chemical synthesis of the amino acid is the more practical method for its industrial manufacturing (9). In the central nervous system, glycine functions as an inhibitory neurotransmitter, particularly in the retina, brainstem, and spinal cord. Chloride enters the neuron through ionotropic receptors when glycine receptors are active, resulting in an inhibitory postsynaptic potential (IPSP) (10). Glycine usually results in death through hyperexcitability and has an oral LD50 of 7930 mg/kg in rats (11).

JUSTIFICATION OF STUDY

The liver plays vital role in the body's metabolic processes. Due to its central role in digestion, its susceptibility in ingestion of toxins is inevitable. The increasing trend of industrialization and the consequent increase in the consumption of heavy metals particularly cadmium form the bases for this investigation. Also, glycine has been reported by many researchers with potentials for anti-oxidants, anti-inflammatory properties amongst others. However, its use in cadmium toxicity is limited hence its choice in this study. This study aimed to investigate the role of glycine in cadmium-induced liver damage in adult wistar rats

Materials and methods

Experimental rats

Thirty Wistar rats weighing between 150 and 170g, purchased from the animal house of the department of Anatomy, University of Benin were used for this study. They were randomly divided into six groups and placed in standard cages with good ventilation (Table 1). The rats were allowed free access to rats feed and water ad libitum. Cadmium and Glycine were administered to rats using orogastric tubes.

Tissue collection, processing and staining and statistical analysis

At the end of the experiment, the rats were anaesthetized using chloroform anesthesia. Five milliliters (5 mls) of blood was collected via cardiac puncture into sterile bottles for analysis of serum ALT, AST, albumin and total protein at the laboratory of the Department of Chemical Pathology, University of Benin Teaching Hospital. Liver tissues were harvested and immediately fixed in 10% formal saline for 72 hours before been processed histologically using Haematoxyline and Eosin staining technique (12). The sections obtained were examined and photomicrographs of sections were taken using Leica DM750 research microscope with an attached digital camera (Leica CC50) at 400x magnification

Results obtained were expressed as Mean ±

SEM (standard error of means). Differences between the means were determined by one-way analysis of variance (ANOVA). Values were considered statistically significant if P value is less than 0.05 ($p < 0.05$). LSD Post Hoc test was used to determine where the significance lay. Statistical package Graph Pad Prism Version 9.0 for Windows (GraphPad Software Inc.) was used to analyze the data obtained in this study.

RESULTS

Fig.1.Changes in body weight (g) of rats in control and treated groups. There was statistically significant decrease ($P<0.05$) in body Weight (g) for rats given 10 mg/kg body weight of cadmium only and a significant increase in body weight for rat given 500 mg/kg body weight of glycine when compared to control.

Fig.2. Changes in serum Alanine Transaminase (ALT) of rats in control and treated groups. There was a statistically significant decrease ($P<0.05$) in ALT of rats treated with 10mg/kg of Cadmium only for 14 days and a statistically significant increase ($P<0.05$) in rats treated with 1000mg/kg of Glycine only for 14 days when compared with control group.

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

There was a statistically significant decrease ($P<0.05$) in AST in rats treated with 10mg/kg of Cadmium for 14 days followed by 500mg/kg of Glycine for 14 days when compared with 10mg/kg of Cadmium only for 14 days group.

There was a statistically significant decrease ($P<0.05$) in Albumin in rats treated with 10mg/kg of Cadmium only for 14 days when compared with control group.

There was a statistically significant increase ($P<0.05$) in Albumin in rats treated with 10mg/kg of Cadmium for 14 days followed by 500mg/kg of Glycine for 14 days when compared with 10mg/kg of Cadmium only for 14 days group.

Fig.5. Changes in serum Total Protein of rats in control and treated groups. There was a statistically significant decrease ($P<0.05$) in total protein in rats treated with 10mg/kg of Cadmium only for 14 days group when compared with control group.

Discussion

Cadmium is a heavy metal element with long biological half-life, slow metabolism and strong toxicity which easily accumulates in the body and thus causing a variety of damages to tissues and organs of the body (13). Cadmium is present in most foodstuffs because of its high rates of soil-to-plant transfer hence human exposure is inevitable (14).

Poisoning caused by long-term chronic accumulation of cadmium has attracted more and more social attention but the pathogenesis of short-term toxicity of high-dose cadmium is not completely clear (15).

In this study, cadmium-treated rats showed significant decrease in body weights when compared to control (Fig 1). However, treatment with glycine prevented the weight loss observed in the cadmium treated group. Cadmium, being a toxic substance, induces a reduction in food consumption in rats. This may account for the significant weight loss observed in the cadmium treated rats. This weight reduction has also been reported in previous study where cadmium treated rats had significant weight reduction when compared to the study control (16). Glycine administration in this study significantly increased the body weight of rats. Glycine is a major component of the body's collagen and helps prevent muscles wasting and stimulate muscle growth, an effect that may contributes to weight gain in the glycine only treated groups

Cadmium exposure is associated with a variety of liver conditions. Cadmium interacts with multiple aspects of liver function, mainly through heavy metal binding proteins called metallothioneins. Acute and chronic cadmium-induced hepatotoxicity is well-known in animal

models of liver failure. In mouse models of chronic cadmium-induced hepatotoxicity, nonspecific chronic inflammation, granulomatous inflammation, apoptosis, and liver cell regeneration have been noted (17). In this study, cadmium treatment in rats significantly affected the liver's function. The serum ALT level was significantly impaired leading to a decrease in the serum level of ALT while the serum level of AST was significantly elevated. Changes in serum ALT and AST are important enzyme indicators of liver injury particularly in the Non-alcoholic fatty liver disease. Their elevation is often related to injury due to inflammatory changes in the liver. Previous study reported a significant association between blood cadmium concentration and serum AST and ALT levels (18). The low level of ALT in this study could be related to renal involvement in cadmium treatment as patients with kidney disease have been reported with low serum ALT level (19). However, further studies are required to elucidate this. Cadmium effect on liver function was also evident in the serum level of albumin by significantly reducing Serum level of albumin. Albumin is the most abundant plasma protein produced in the liver. Low levels of serum albumin may indicate damage to the liver or liver failure. Low serum albumin could also be related to cadmium effect on the kidneys. It has been reported that cadmium ingestion reduces glomerular filtration rate (GFR), reduces albumin reabsorption in the renal tubules thus increasing tubular proteinuria with resultant increase of albumin in the urine (20). The serum total protein was significantly reduced with cadmium treatment when compared with the control group in this study. This significant reduction in total protein could also be related to the reduction in serum albumin level which makes up the majority of the plasma protein in the body. These effects induced by cadmium were however reversed in rats following treatment with glycine.

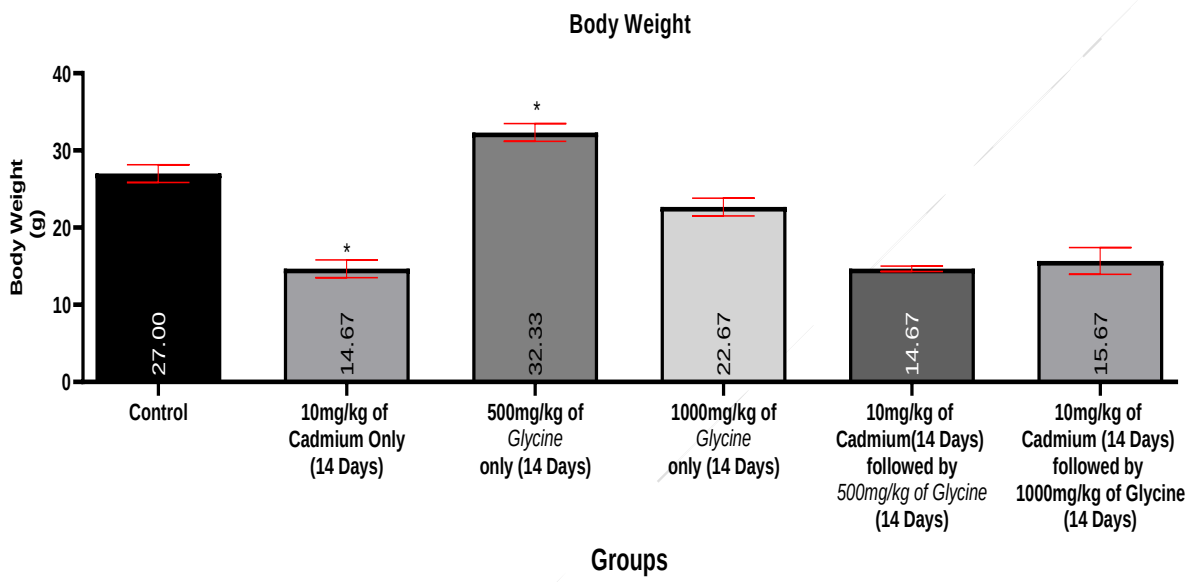
The liver histological findings in this present study showed normal liver architecture in control liver with hepatocytes, sinusoids and central vein clearly indicated (Plate 1). The cadmium treated group showed features of necroinflammation evidenced by area of zonal necrosis and infiltration of inflammatory cells in cadmium exposed rat liver (Plate 2). Thus supporting the inflammatory potential of cadmium by previous researchers. These effects were however reversed following treatment with glycine (Plate 5 and 6). It was also observed that glycine treatment induced lymphocytes mobilization in rat's' liver (Plate 3 and 4). This is an indication that in addition to anti-inflammatory properties of glycine reported by previous studies, glycine also possesses immunologic potentials.

Conclusion

Glycine possesses anti-inflammatory and ameliorative properties against cadmium-induced liver toxicity. This is evidenced by a glycine-induced reversal of the ALT, AST, Albumin, Total protein derangements and histological features following cadmium treatment.

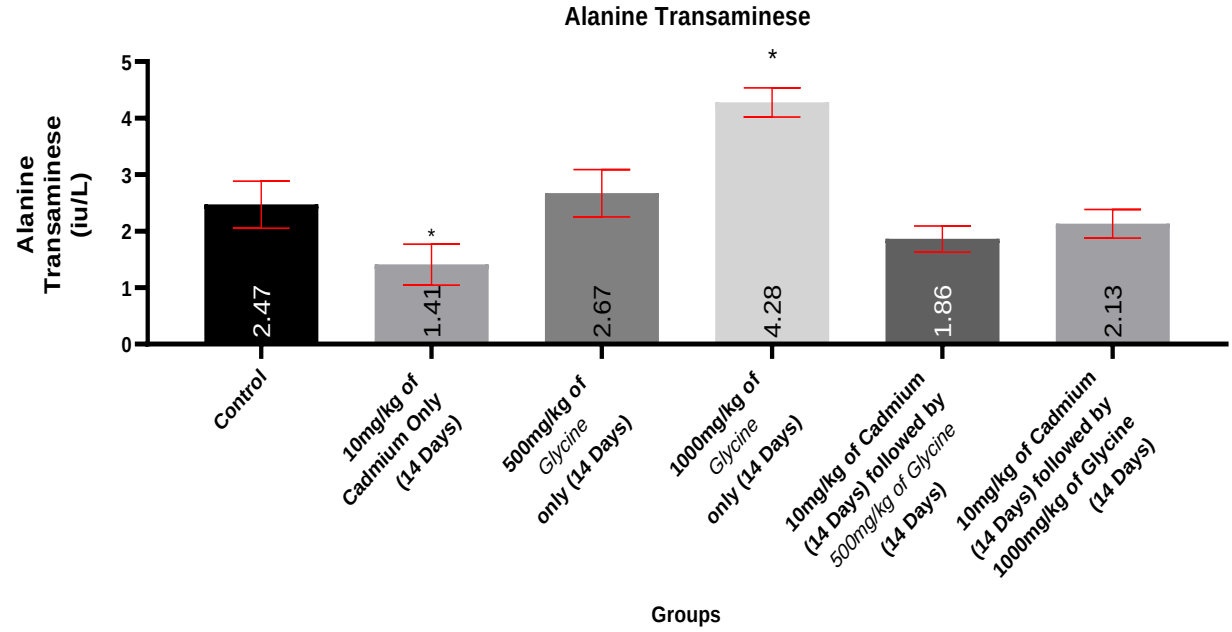
Table 1: Experimental Design

GROUPS	REGIMEN
Group A	Served as control and were fed with Animal feed (Topline Grower Mash by Premier feed Co. Ltd, Ibadan Oyo State) and water.
Group B	Received 10mg/kg body weight of cadmium for 14 days.
Group C	Received 500mg/kg body weight of glycine for 14 days.
Group D	Received 1000mg/kg body weight of glycine for 14 days.
Group E	Received 10mg/kg body weight of cadmium for 14 days and 500mg/kg body weight of glycine for 14 days
Group F	Received 10mg/kg body weight of cadmium for 14 days and 1000mg/kg body weight of glycine for 14 days



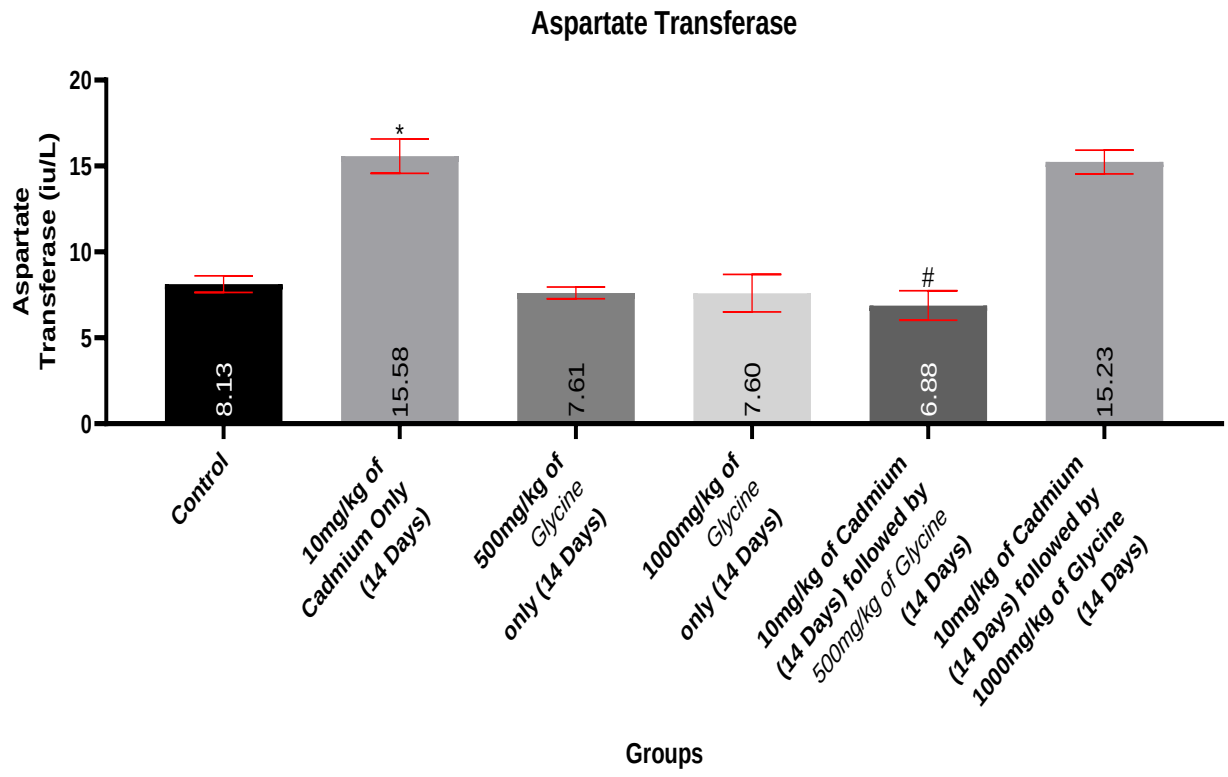
*Represent test group compared to control (P<0.05)

Fig.1.Changes in body weight (g) of rats in control and treated groups

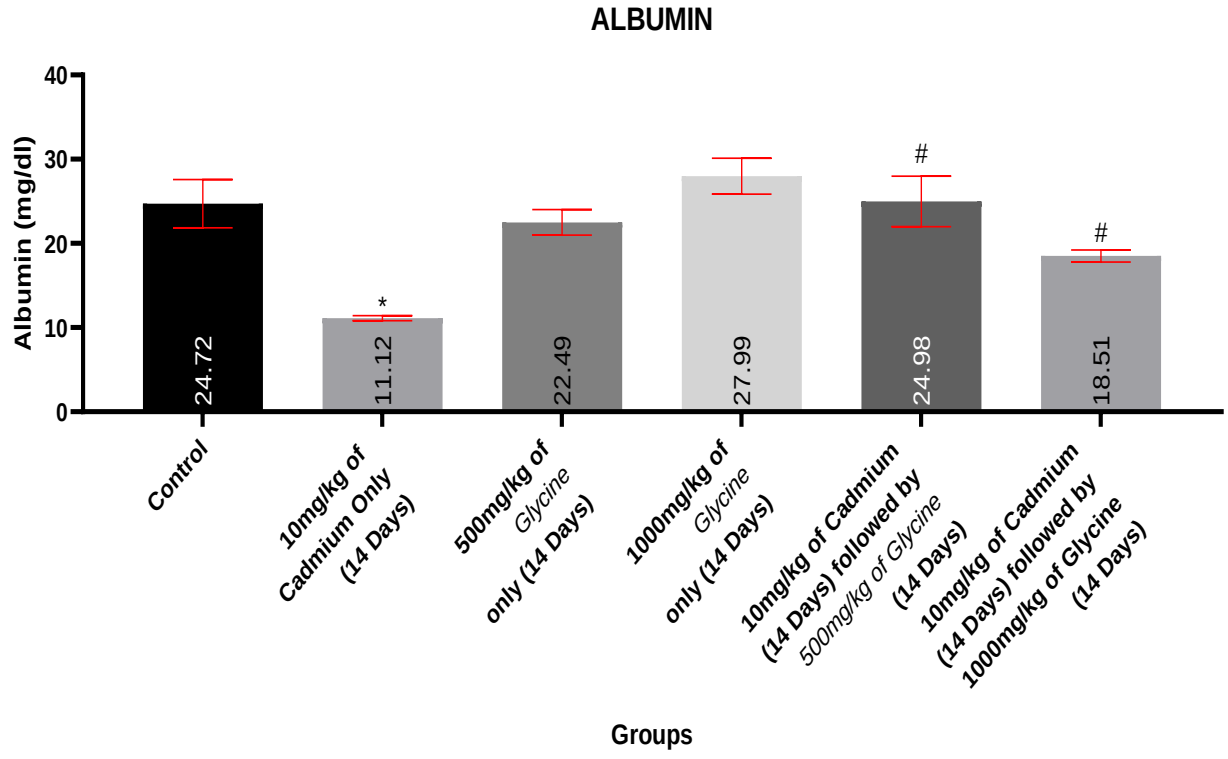


*Represent test group compared to control (P<0.05)

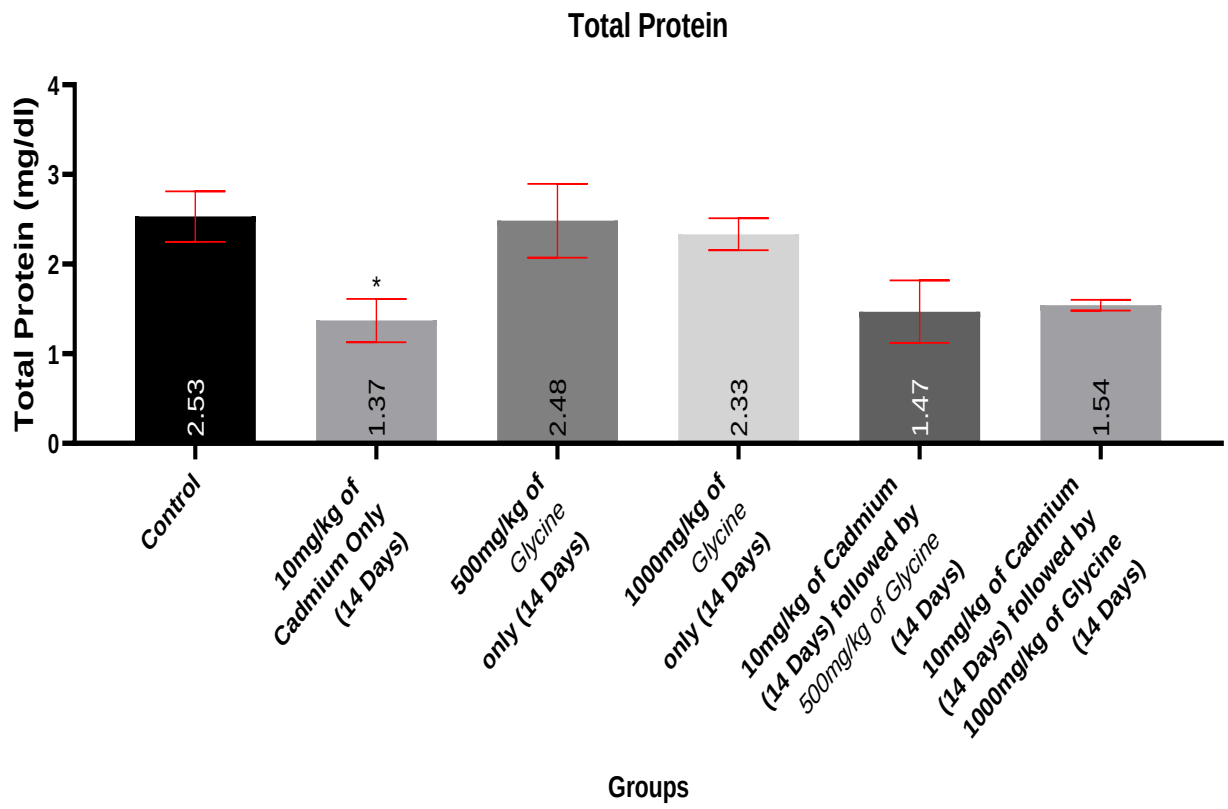
Fig.2. Changes in serum Alanine Transaminase (ALT) of rats in control and treated groups



*Represent test group compared to control (P<0.05)
#Represents test group compared to 10mg/kg of Cadmium only (P<0.05)
Fig.3. Changes in serum Aspartate Transaminase (AST) of rats in control and treated groups



*Represent test group compared to control (P<0.05)
#Represents test group compared to 10mg/kg of Cadmium only (P<0.05)
Fig.4. Changes in serum Albumin level of rats in control and treated groups



Represent test group compared to control (P<0.05)
Fig.5. Changes in serum Total Protein of rats in control and treated groups

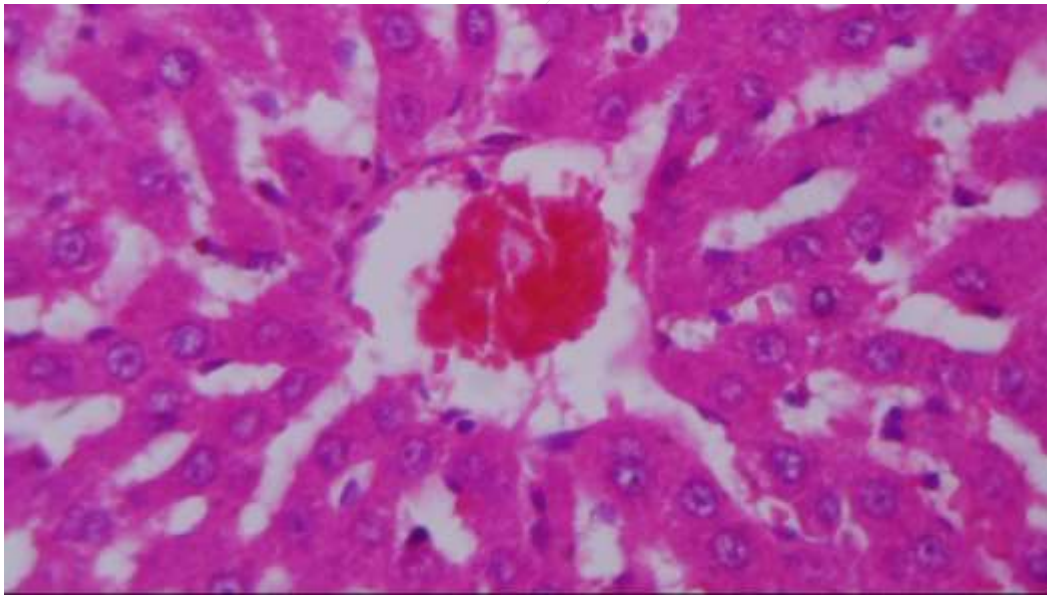


Plate 1: Rat liver. Control. Composed of normal liver architecture: hepatocytes (HE), sinusoids (SI), central vein (CV) H & E 400x

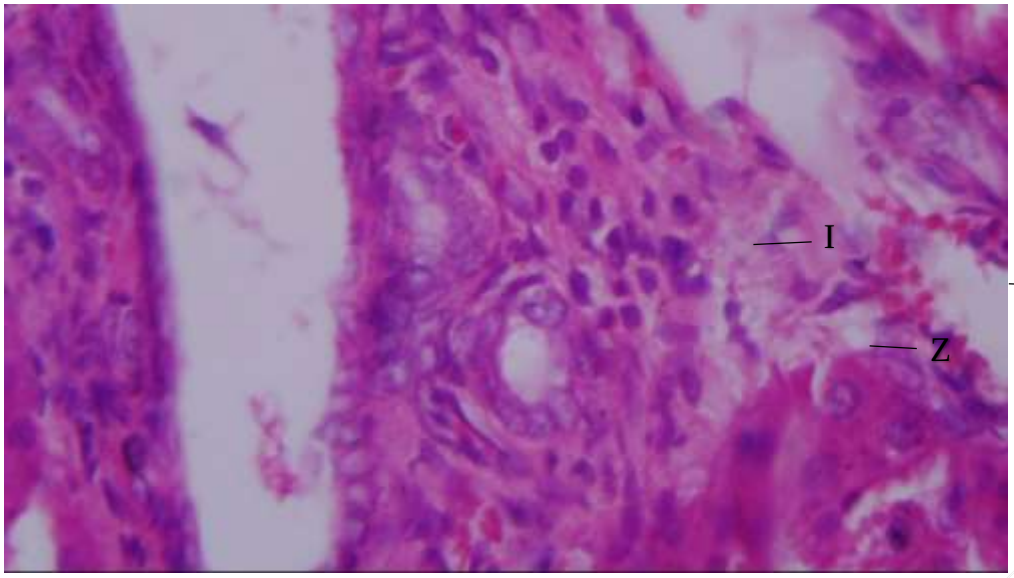


Plate 2: Rat liver given 10mg/kg of Cadmium only showing: vascular congestion (VC), zonal necrosis (ZU), heavy periportal infiltrates of inflammatory cells (IC) (H&E 400x)

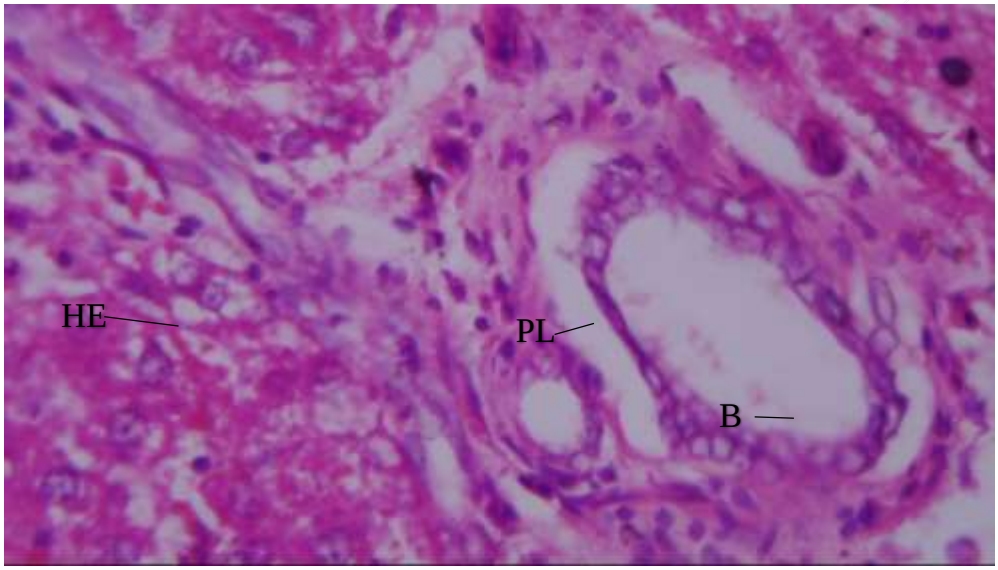


Plate 3: Rat liver given 500mg/kg of Glycine showing normal architecture: hepatocytes (HE), periportal mobilization of lymphocytes (PL), bile ducts (BD) (H&E x 400)

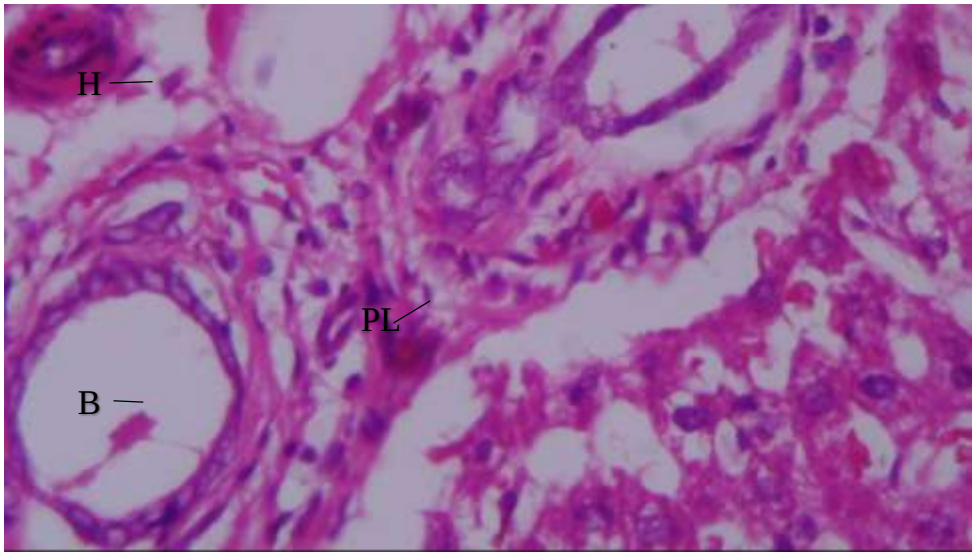


Plate 4: Rat liver given 1000mg/kg of Glycine only showing normal tissue: hepatocytes (HE), hepatic artery (HA), periportal mobilization of lymphocytes (PL), Bile ducts (BD) (H&E 400x)

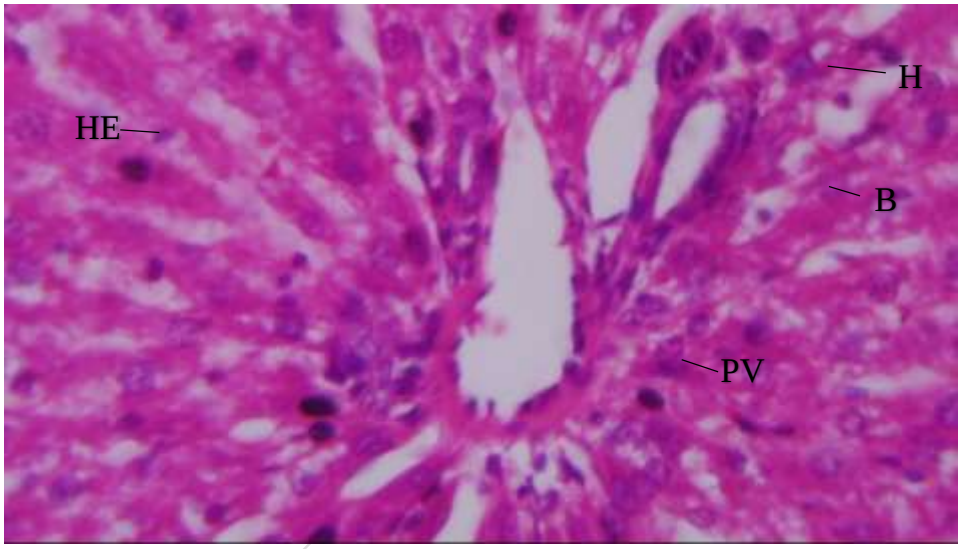


Plate 5: Rat liver given 10mg/kg of Cadmium + 500mg/kg of Glycine only showing normal architecture: hepatocytes (HE), portal vein (PV), hepatic artery (HA), bile ducts (BD) (H&E 400x)

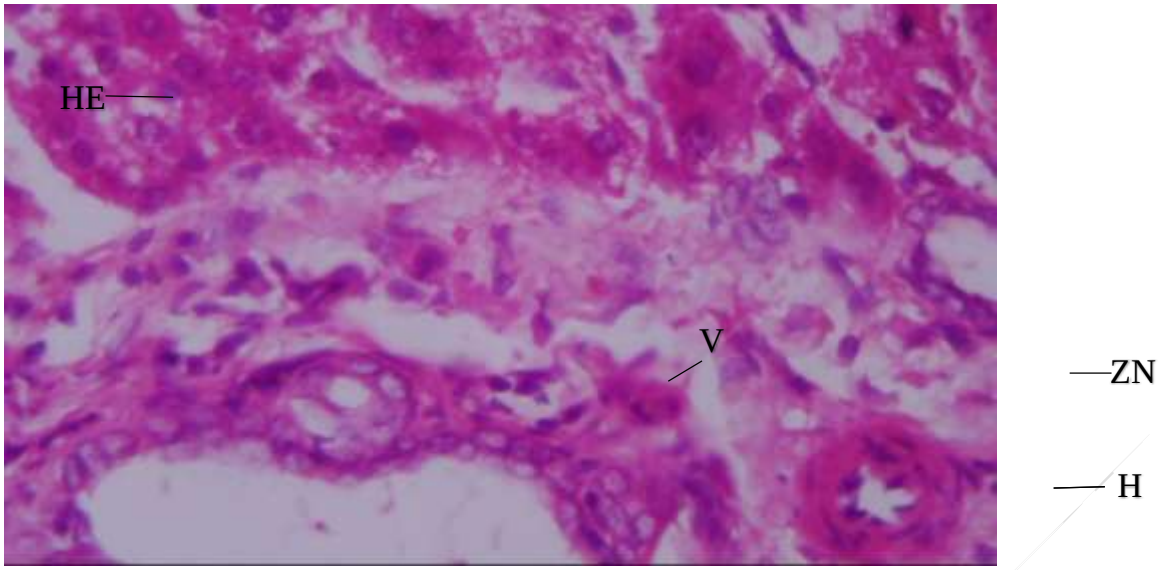


Plate 6: Rat liver given 10mg/kg of Cadmium + 1000mg/kg of Glycine only showing: normal hepatocytes (HE), zonal necrosis (ZN), vascular ulceration (VU), hepatic artery (HA) (H&E 400x)

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