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Majid Shafi
Division of Veterinary Pathology Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir, Jammu & Kashmir, India

Showkeen Muzamil
Division of Veterinary Biochemistry, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST Kashmir, Jammu & Kashmir, India

Sami Jan
Post Graduate Student, GMC Srinagar, Kashmir, Jammu & Kashmir, India

Omer Khalil
Division of Veterinary Pathology, Faculty of Veterinary Sciences and Animal Husbandry, GADVASU, Ludhiana, Punjab, India

Shabia Shabir
Islamic University, Jammu & Kashmir, India

Masood Saleem
Division of Veterinary Pathology Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir, Jammu & Kashmir, India

Shayaib Ahmad
Division of Veterinary Pathology Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir, Jammu & Kashmir, India

Mir Nadeem
Division of Veterinary Microbiology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST Kashmir, Jammu & Kashmir, India

Anisa
Division of Veterinary Pathology Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir, Jammu & Kashmir, India

Showkat Ahmad Shah
Division of Veterinary Pathology Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir, Jammu & Kashmir, India

Corresponding Author:
Showkat Ahmad Shah
Division of Veterinary Pathology Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir, Jammu & Kashmir, India

Exploring Hepato-pathological hallmarks for the surveillance and investigation of metformin induced toxicity in Wister rats

Majid Shafi, Showkeen Muzamil, Sami Jan, Omer Khalil, Shabia Shabir, Masood Saleem, Shayaib Ahmad, Mir Nadeem, Anisa and Showkat Ahmad Shah

Abstract
Background: Diabetes is frequently treated with the medication metformin and its chronic use may lead to hepatopathy in humans as well as in animals.
Aim: The purpose of this study was to assess the effects of long-term Metformin administration on the rat liver's structure and functions.
Material and Methods: In this study, Twenty Wister rats were used in this investigation, and they were randomly split into two groups of ten each. The first group served as the control and the second group got metformin hydrochloride at a dose of 30 mg/kg body weight/day for a period of twenty one straight days. At the end of study period, all rats were sacrificed to analyze the hematological, biochemical and the path morphological changes observed in the liver of rats.
Conclusion: The outcomes showed that metformin produces significant liver dysfunctions in rats, which were supported by biochemical analyses and liver histology.

Keywords: Hepatic dysfunction, histopathology, hematology, biochemistry, metformin

Introduction
Diabetes is a chronic condition brought on by either insufficient insulin production by the pancreas or inefficient insulin utilization by the body. The pancreas produces the hormone insulin, which regulates the level of glucose in the bloodstream at any given time. Glucose is also stored in the liver and muscles. In addition to this, Insulin is a key player in controlling the metabolism of proteins, lipids, and carbohydrates in both human and animal bodies. Type 2 diabetes prevalence has risen to epidemic levels, increasing the risk of cardiovascular illnesses in people and animals. Metformin is a commonly prescribed medication for the management of Type 2 diabetes which lowers blood glucose levels by preventing gluconeogenesis in hepatocytes and reducing intestinal glucose absorption (Viollet *et al*., 2012) [20]. Metformin was originally developed from glargine found in the plant Galega officinalis (Bailey and Day, 2004) [3]. Metformin is a unique anti-diabetic drug which plays an important role in the controlling blood glucose level and increases insulin sensitivity (Brown *et al*., 2008) [4]. Metformin inhibits the mitochondrial respiratory chain in the liver on a molecular level, which activates AMPK, lowers cAMP, and reduces the production of gluconeogenic enzymes (Rena *et al*., 2017) [24]. Besides its previous use, it was recently used for the treatment of cancer (Mobasher, *et al*., 2021) [13] and pre-clinical trials in patients suffering with COVID-19 (Samuel *et al*., 2021) [25].
The liver is the largest solid organ in the body, regulates blood clotting, keeps blood sugar levels in a safe range, and removes toxins from the blood. Hepatic pathology is a typical feature of many disorders that practitioners and researchers routinely encounter. Although the liver is frequently implicated in illness processes that also affect a variety of other organ systems, some disorders are exclusive to the liver.
Based on the above listed biological effects of Metformin, this study was conducted to evaluate the role of long-term treatment with Metformin on the structure and functionality of liver in experimental rats.
Keeping in view the paucity of information regarding the toxicity of the metformin, this study was undertaken in Wister rats with the following objectives:
1. To study the hematological and biochemical alterations in metformin induced toxicity in Wister rats.

2. To study the clinical and pathomorphogical effects of metformin induced toxicity in Wister rats.
3. To compare the organ weight index between the control and the intoxicated group of Wister rats.
4. To evaluate the effect of Metformin treatment on the oxidative stress biomarkers in the rat liver tissues.

Materials and Methods

Chemicals

In the Srinagar district of Jammu and Kashmir, we purchased commercially accessible biochemical kits and metformin hydrochloride from a local market

Animals

Wister rats weighing between 170 and 200g were purchased for this experiment from the CSIR Jammu animal house. The animals were housed at room temperature (30oC) in ventilated cages. The rats were given unlimited access to tap water and regular pelleted animal food. Protein makes up 21% of the average diet, followed by lipids at 3.2%, carbs at 68.2%, and fibers at 3.44%. Rats were handled and treated in compliance with National Institutes of Health recommendations for the care and management of laboratory animals. The Faculty of Veterinary Sciences, SKUAST-Kashmir's institutional ethics committee accepted the experimental protocol.

Study Design

In this study, toxicopathological responses to metformin were examined in the Wistar rats. The Twenty Wistar rats were randomly divided into two groups for this invetigation, with ten rats kept in each group. Prior to the trial, the wistar rats were given a two-week acclimation period. The first group acted as the control, and the second group received oral gavage metformin hydrochloride for 21 straight days at a dose of 30 mg/kg body weight. The dose rate was selected on the basis of pervious literature as reported by Oluwatosin *et al*., 2012 [19].

Clinicopathological studies

The Clinical symptoms in the test animals were observed throughout the entire investigation. Blood samples were taken from the animals and kept in sterile vials for clinicopathological studies. For haematological research, only half of the blood that had heparin injected as an anticoagulant was used. After the serum was separated, the remaining blood was used for biochemical analysis and was kept at -20 °C until needed.

Hematological studies

In this trial, the subsequent hematological investigation was performed manually.

1. Hemoglobin
2. Total Erythrocyte count
3. Total leukocyte count
4. Hematocrit

Liver function test

The liver function test and other blood biochemical parameters were estimated by using Olympus biochemistry analyser with compatible kits

1. Alanine transaminase (ALT)
2. Aspartrate transaminase (AST)
3. Alkaline Phosphatse (ALP)
4. Total Protein
5. Albumin
6. Globulin

Determination of Triglycerides, Cholesterol, HDL, LDL and VLDL levels in the sera of rats

All of the rats were slaughtered at the end of the experiment, and blood samples were taken. The Sera were separated and kept in aliquots at -70 °C until the colorimetric method was used to estimate triglycerides, total cholesterol, LDL cholesterol, VLDL cholesterol, and HDL cholesterol.

Relative weight of different organs

At the conclusion of the study period, the animals in each group had their internal organs removed. These organs were all lightly blotted, weighed, and the relative weights were computed in accordance with Garg's (2004) [8] instructions. By dividing the organ weight of each animal by its body weight, one can determine the relative organ weight.

Tissue Processing for Histopathological Examination

The Rat liver tissue samples were taken from the animals and preserved in 10% formalin. Following the completion of the tissue processing in various degrees of alcohol and xylene, the paraffin blocks were created. Using a microtome, sections of around 5 m were cut from paraffin blocks, and as advised by Lille, 1954, staining was carried out using hematoxylin and eosin. In order to see cellular damage, the tissue pieces are next seen under a light microscope.

Statistical analysis

All data are presented as mean (M)±standard deviation (SD) and statistical differences between means were determined by one-way ANOVA

Results

Clinical Signs

The intoxicated animals showed slow growth, weight loss, dullness, depression, emaciation, dehydration and increased salivation. In rats, the clinical symptoms became more noticeable one week after the intoxication. The intoxicated animals displayed lethargy, diarrhea, lack of appetite, thirst, and labored respiration during this research period. After the first week, the respiratory motions slowed down and the breathing became harder. Over the course of the trial, a drop in body weight growth was seen. The affected animal showed sternal recumbency, convulsion, lacrimation from the eyes, rough body coat, excessive salivation, diarrhoea, restlessness, emaciation, and polydipsia were among the clinical signs seen in the third week of intoxication. From the start of the trial until it was over, the affected animals had an increase in body temperature. At the conclusion of the trial, the intoxicated rats that had persisted for a few days had displayed stunted growth, watery feces, muscle weakness, stumbling gait, and lack of stimulus response. The affected animals stood still for a considerable amount of time with their eyes closed.

Hematology

The findings, as shown in Table 1, indicated that there was no significant difference between the study groups in terms of hemoglobin (Hb) level, total red blood cell (RBC) counts, hematocrit (Hct) percentage, or total white blood cells (WBCs) count at the conclusion of the experimental period.

Table 1: Mean ± S.D. of different hematological parameters in Wister rats after intoxicated with Metformin

Groups	Hb (g/dL)	RBCs (x106 /µL)	Hct (%)	WBCs (x103 /µL)
Control	11.31±0.56	9.73±0.42	42.4±2.1	12±0.8
Treatment	11.60±0.41	9.62±0.15	41.8±2.5	12±0.6

Means of various Hematological parameters does not differ significantly

Serology

At the conclusion of the experiment, the animals in the metformin treatment group showed the greatest levels of AST, ALT, and ALP in the serum when compared to the control group.

Table 2: Mean ± S.D. of serum AST ALT and ALP (U/L) in Wister rats after intoxicated with Metformin

Groups	AST	ALT	ALP
Control	67.5 ^{a}±11.83	36.5 ^{a}±3.25	145.27 ^{a}±23.68
Metformin	125.8 ^{b}±35.71	76.5 ^{b}±8.93	205.30 ^{a}±73.60

Means of various biochemical parameters differ significantly between the groups

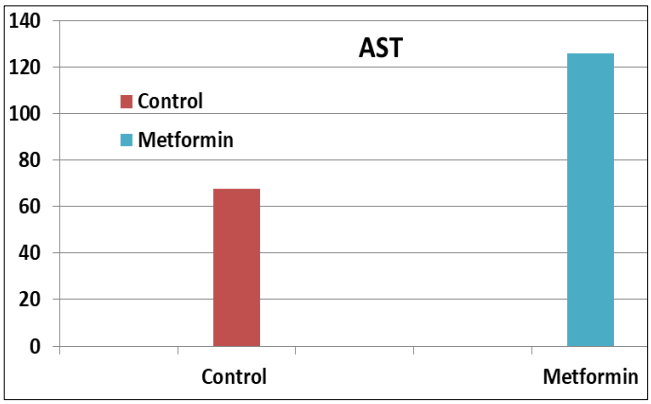

Fig 1a: Effect of Metformin intoxication on Aspartate transaminase (AST) level in Wister rats

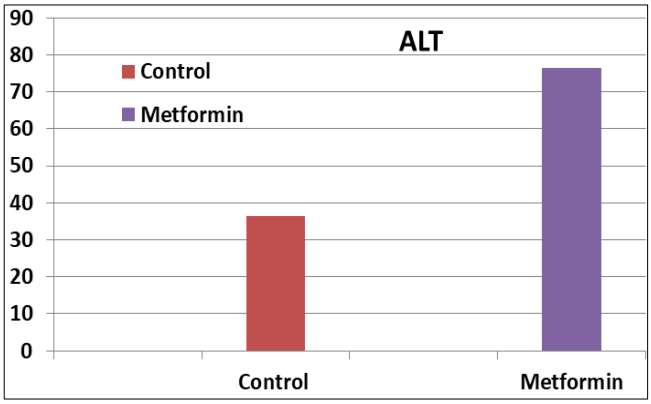

Fig 1b: Effect of Metformin intoxication on Alanine transaminase (ALT) level in Wister rats

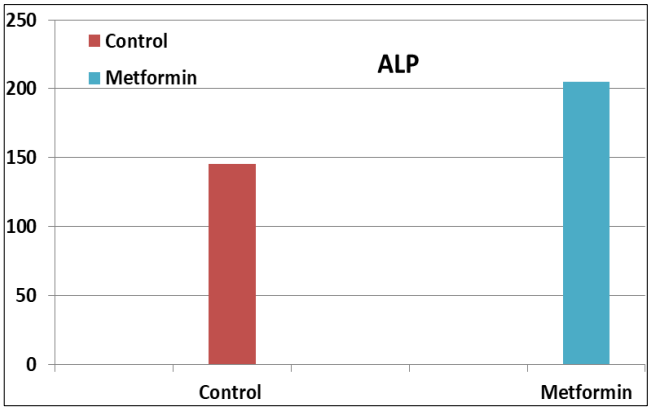

Fig 1c: Effect of Metformin intoxication on Alkaline Phosphatase (ALP) level in Wister rats

The other biochemical parameters like Total Protein, Albumin and Globulin at the end of experimental period were raised in metformin intoxicated animals when compared to control group as shown in table 3.

Table 3: Mean ± S.D. of serum Total protein, Albumin and Globulin levels (gm/dl) in Wister rats after intoxicated with Metformin

Groups	Total Protein	Albumin	Globulin
Control	5.17 ^{a}±0.21	2.63 ^{a}±0.24	2.73 ^{a}±0.25
Metformin	6.72 ^{b}±0.40	3.74 ^{b}±1.62	3.75 ^{b}±0.31

Means of different biochemical parameters differ significantly between the groups

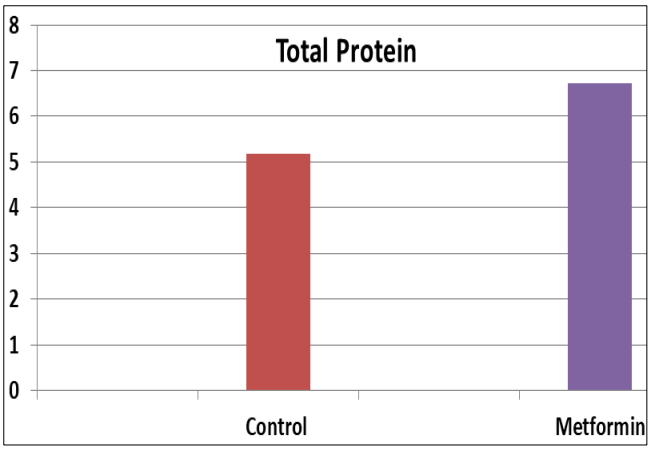

Fig 2a: Effect of Metformin intoxication on total protein level in serum of Wister rats

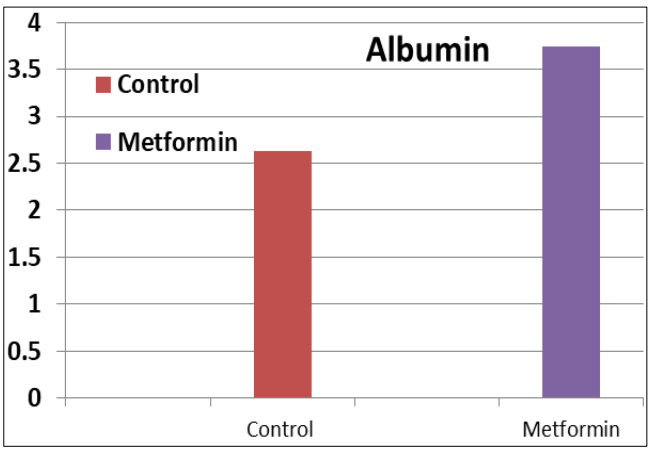

Fig 2b: Effect of Metformin intoxication on Albumin level in serum of Wister rats

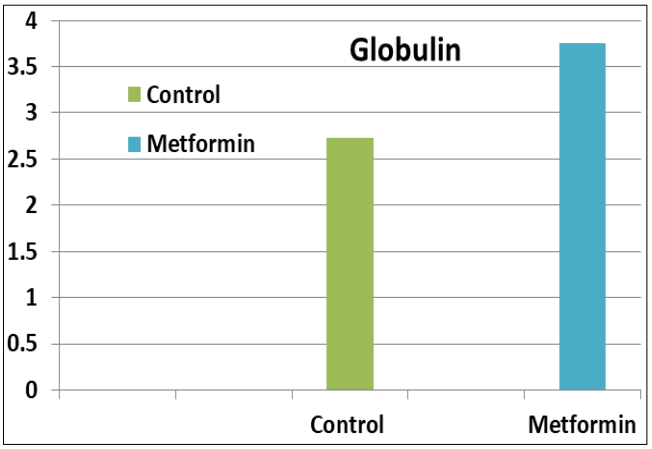

Fig 2c: Effect of Metformin intoxication on globulin level in serum of Wister rats

Effect of Metformin on the lipids profile in Wister rats

When compared to the levels in the control group at the end of the trial, the results indicated that the metformin-intoxicated rats had lower levels of cholesterol, triglycerides, LDL, HDL, and VLDL.

Table 4: Cholesterol, triglycerides (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL) levels in the different groups under the study.

Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	42.3±1.5[a]	54.00±2.35[a]	37.3±1.2 [a]	11.00±1.0 [a]	15.00±1.0 [a]
Metformin	40.0±1.0[b]	50.00±1.26[b]	34.3±1.4 [b]	8.00±1.0 [b]	13.00±1.0 [b]

Means of different biochemical parameters differ significantly between the groups

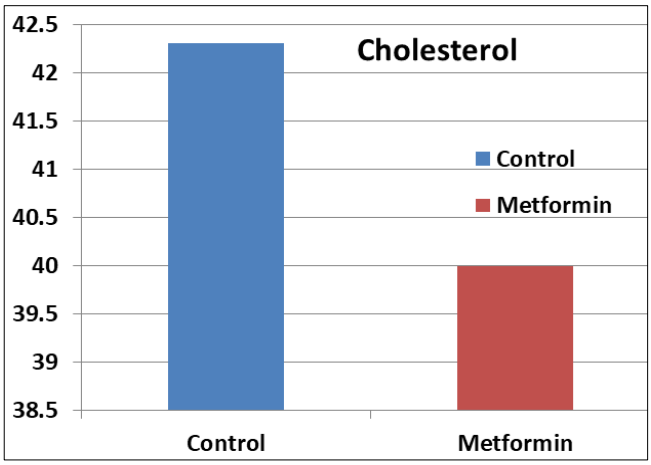

Fig 3a: Effect of Metformin intoxication on cholesterol level in Wister rats

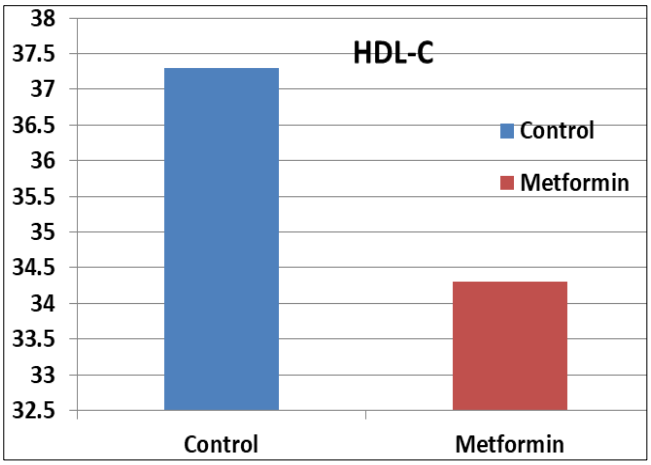

Fig 3c: Effect of Metformin intoxication on high density lipoprotein level in Wister rats

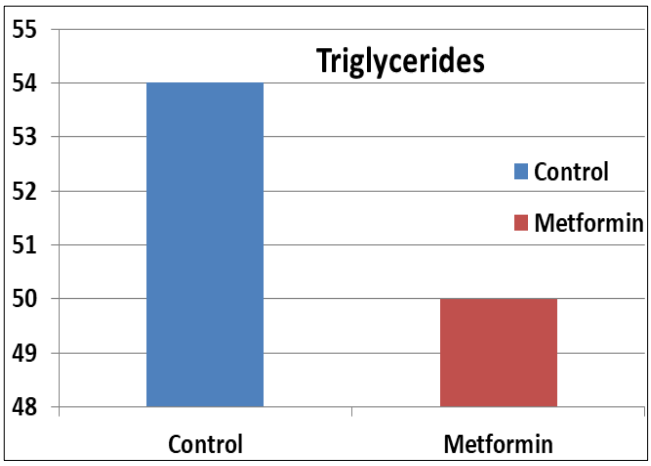

Fig 3b: Effect of Metformin intoxication on triglycerides level in Wister rats

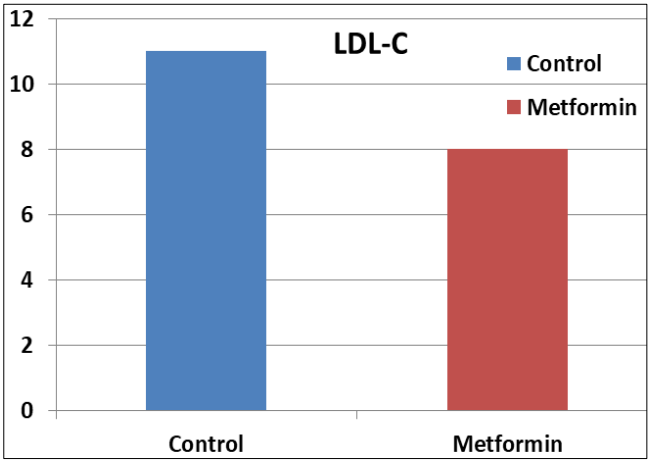

Fig 3d: Effect of Metformin intoxication on low density lipoprotein level in Wister rats

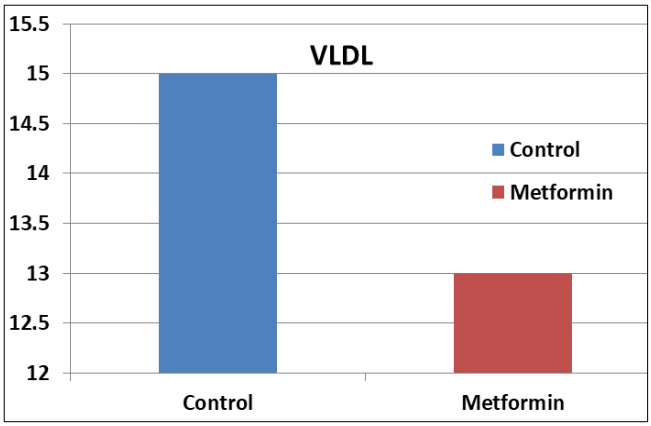

Fig 3e: Effect of Metformin intoxication on very low density lipoprotein level in Wister rats

Gross Pathology: The liver initially seemed normal, but as it progressed, it became darker, enlarged, and congested. The intoxicated rats have enlarged livers that also had petechiaeal hemorrhages on their surface. The livers were normally firm to the touch and had white necrotic foci on their outer surface. The most prevalent gross abnormalities in the liver were a yellowish discolouration and ecchymotic hemorrhages at day7. The intoxicated rats have had rounded edges livers, and were a dark in color as compared to normal one. In certain cases, the hemorrhages took the form of tiny, pinpoint-sized petechiae, ecchymosis, or suffusions. A significant amount of blood has occasionally leaked from the liver's cut surfaces.

On physical examination, the rats that were slaughtered at day 14 had livers that were pale, swollen, and friable. Hepatic congestion and hemorrhages were also found in the individuals that were seriously impacted. Liver often had rounded margins, giving the impression that it was spherical in shape. The livers were simple to cut, and the cut surface had an oily, bulged appearance. The livers were inflated with rounded margins, and in some cases the swellings were significant and gave the surface of the liver a granular look. The damaged rats' livers also had a lot of yellow spots and red hemorrhagic foci that were evenly spaced across their surfaces. On the surface of the liver, there were occasionally big irregular necrotic foci and hemorrhagic streaks.

The necrotic foci on the surface of liver were more pronounced at the end of the study period, and the organ showed up as pale, discolored regions that stood out noticeably from the surrounding healthy tissue. These pale patches were typically multifocal and small in size, ranging from a pinhead to a point, in the majority of the instances. When the condition was severe, the liver's surface was covered in severe hemorrhages and had rounded boarders. The liver capsule appeared thickened and closely adherent with other adjacent organs. In a few cases either single or multiple large, diffuse areas of necrosis were also observed on the surface of liver. In most of the cases necrotic areas were usually surrounded by a brighter zone and were mostly associated with areas of hemorrhage. The focal necrotic areas were often associated with the presence of petechiae at the periphery of the liver of intoxicated rats.

Histopathology

Liver: The microscopic analysis of the control group's liver sections revealed that the hepatocytes had a typical architecture that emanated from the central vein. The liver strands alternated with regular blood sinusoids, which are surrounded by endothelial cells and unique Kupffer cells that are phagocytic in nature.

When the metformin-treated animal was sacrificed on day 5, the hepatic architecture, blood sinusoids, central veins, and degenerating hepatocytes with pyknotic nuclei were all clearly disorganized. The intoxicated rats' liver sections also showed lysis of the hepatic cytoplasm, increased vacuolation, and some cells that were entirely occupied by enormous vacuoles that gave them a balloon-like appearance. At this point, the majority of the liver segment showed proliferative alterations, which were defined by the infiltration of neutrophils and leukocytes, primarily mononuclear cells, as well as the proliferation of fibroblasts in various locations throughout the liver parenchyma. The hepatocellular degeneration, necrosis, and cellular enlargement as well as thickening of the blood vessel wall accompanied these alterations.

The animal was sacrificed on day 10 revealed massive extravasations of erythrocytes from the affected livers. The damaged liver's hemorrhagic region is different sizes and shapes. The hemorrhagic regions could be multifocal and affect the entire liver parenchyma in some cases. Hepatocytes near the hemorrhage showed degenerative alterations including cellular enlargement with cloudy cytoplasm, nuclear pyknosis, karyorhexsis, and karyolysis. The liver hemorrhage was particularly noticeable around the major vein. The intoxicated rats' liver slices also showed clogged blood arteries, enlarged hepatic sinusoids, and overall thicker liver capsules due to neutrophil infiltration. In addition to this, theHepatocyte deterioration to varying degrees was seen in the liver throughout the research period. The perivascular areas showed evidence of hepatitis, which was characterized by neutrophil infiltration and destruction of hepatocytes. In a few instances, the walls of blood vessels thickened and mononuclear cells infiltrated the portal areas.

At day 15, the livers of metformin-treated animals that had been sacrificed exhibited various sized focal regions that were characterized by mononuclear cell infiltration, degeneration, clogged hepatic sinusoids, and sizable areas of coagulative necrosis in the liver parenchyma. The presence of eosinophilic cytoplasm and nuclear alterations such as pyknosis, karyorhexis, and karyolysis were characteristics of necrotic hepatocytes. The proliferation of fibrous tissue was generally localized, as seen around the hepatic lobules, and in some areas it was so severe that it totally replaced the liver parenchyma.

Hepatocytes in the affected area were severely necrosed, hypertrophie, and had malformed nuclei. Furthermore, the organization of the hepatic cord is severely disrupted, making it impossible to identify the hepatocytes other than by their degenerated nuclei, which are encircled by tiny masses of cytoplasm. At this point, the majority of cases showed enlarged hepatocytes, cytoplasm that appeared granular, and increased eosinophilia. The liver sections' microscopic analysis also revealed a dilaled central vein, disrupted hepatocytes with rounded edges, and disorientation. The perivascular zone, which stood out clearly from the surrounding sections, was particularly affected by the deterioration. Additionally, sinusoidal dilatation and a significant number of clear gaps in the form of vacuoles were occasionally seen in the liver sections, which were dispersed throughout the liver parenchyma.

The animals were sacrificed at day 21 revealed damaged hepatocytes, an engorged central vein with RBCs, cellular infiltration, and dilated blood sinusoids in between hepatic

cords. Additionally, liver sections from several of the affected animals showed hepatocytes with pyknoti nuclei with rounded borders. Other histological changes seen in liver sections include pronounced hepatic architectural disarray, vacuolated cytoplasm with defined borders, and karyolysis. Along with hepatocellular deterioration, certain liver sections also showed the existence of many necrotic foci spread throughout the parenchyma. In few cases, liver sections from intoxicated rats showed severe capsular thickening, degenerating hepatocytes, enlarged nuclei, and leukocytic infiltration. The affected rats' liver parenchyma displayed different degrees of hepatocellular deterioration, sinusoidal dilatation, hemorrhage, and venous congestion. The hepatocytes that were impacted showed swelling, rounded corners, and cloudy cytoplasm. Around portal triads, there was a significant infiltration of leukocytes, primarily neutrophils and lymphocytes.

Histopathology

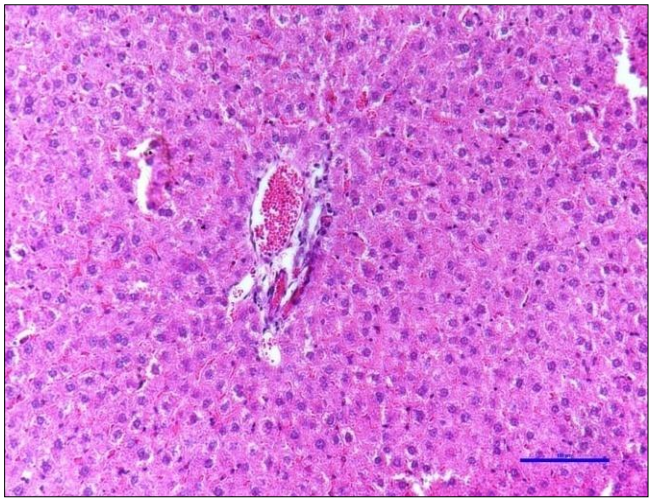

Fig 4: Photomicrograph revealing central vein congestion with perivascular infiltration of mononuclear cells and Neutrophils

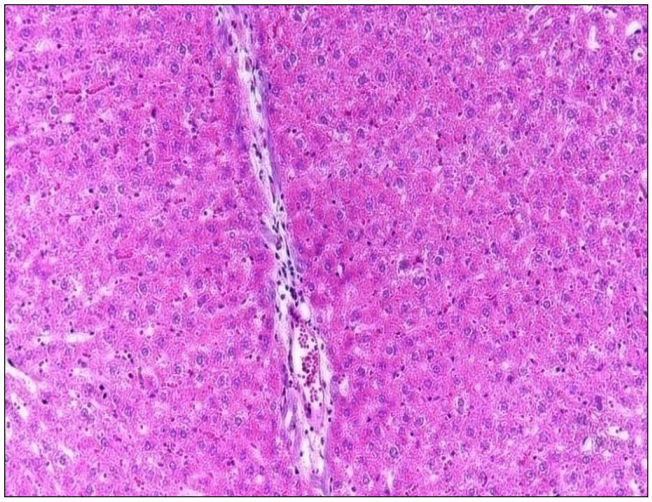

Fig 5: Photomicrograph revealing hepatocellular degeneration with neutrophilic infiltration

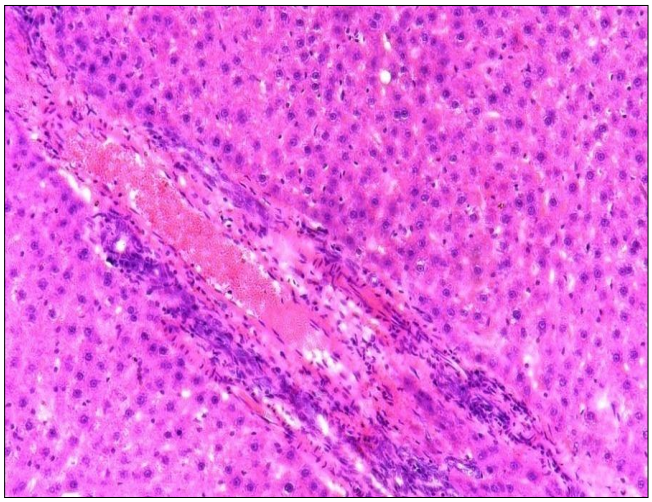

Fig 6: Photomicrograph revealing engorgement of blood vessels with perivascular infiltration

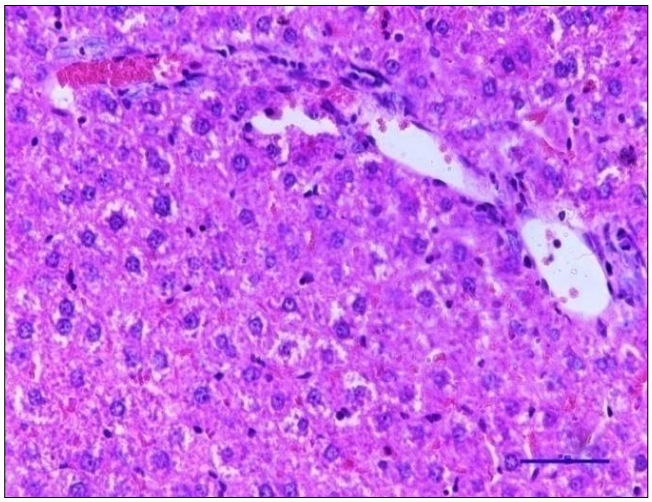

Fig 7: Photomicrograph revealing necrosis and individualization of hepatocytes

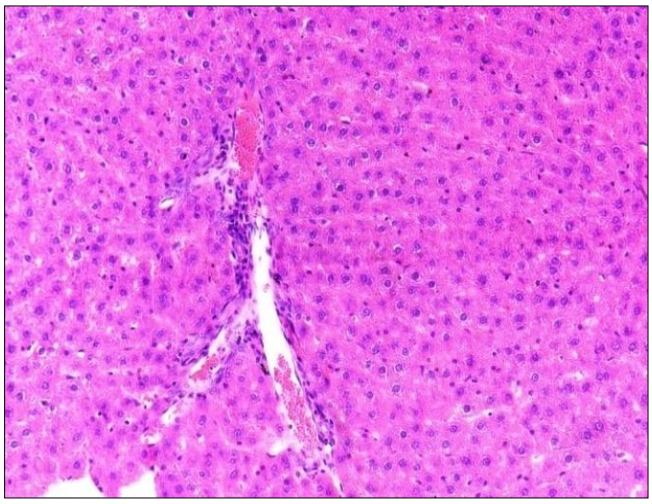

Fig 8: Photomicrograph revealing hepatitis in the perivascular areas

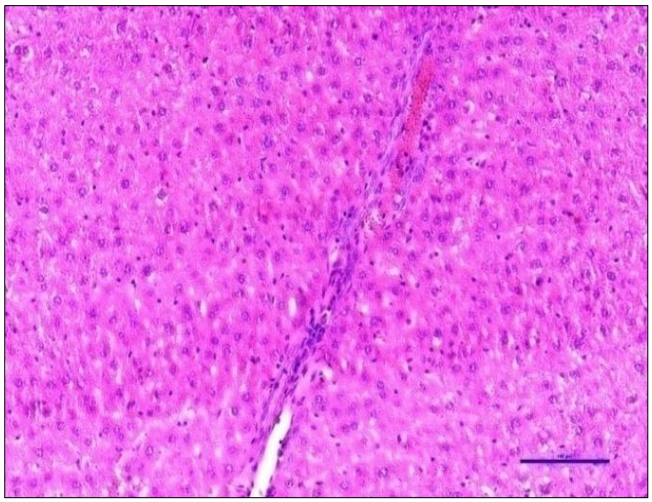

Fig 9: Photomicrograph revealing congested blood vessel and perivascular infiltration

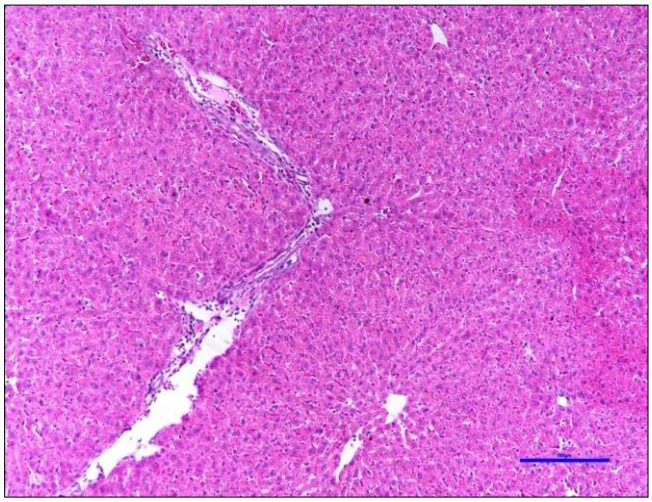

Fig 10: Photomicrograph revealing engorged blood vessel, necrosis and cellular infiltration

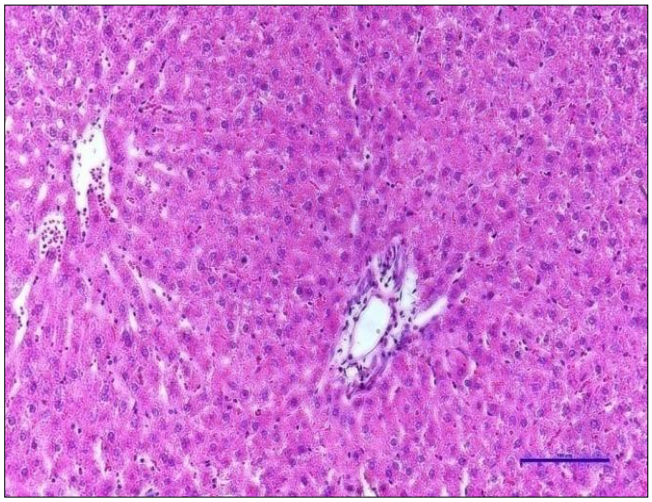

Fig 11: Photomicrograph revealing thickening of the wall of blood vessel along with hepatic degeneration

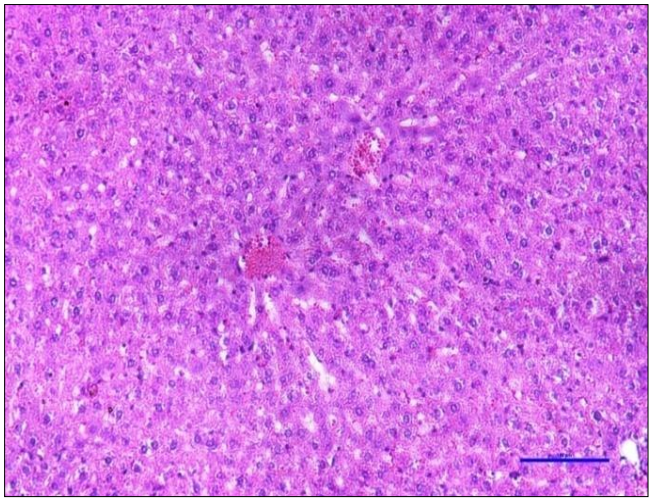

Fig 12: Photomicrograph revealing central vein congestion along with the disruption of hepatic architecture

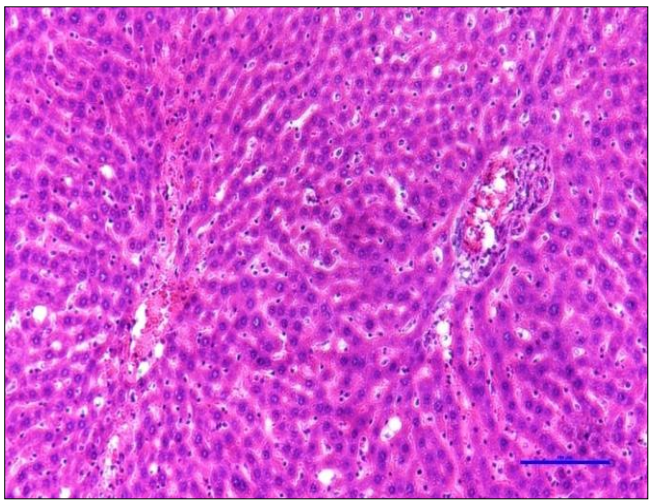

Fig 13: Photomicrograph revealing degenerated with darkly stained nuclei of hepatocytes, congested central vein and Perivascular cellular infiltration

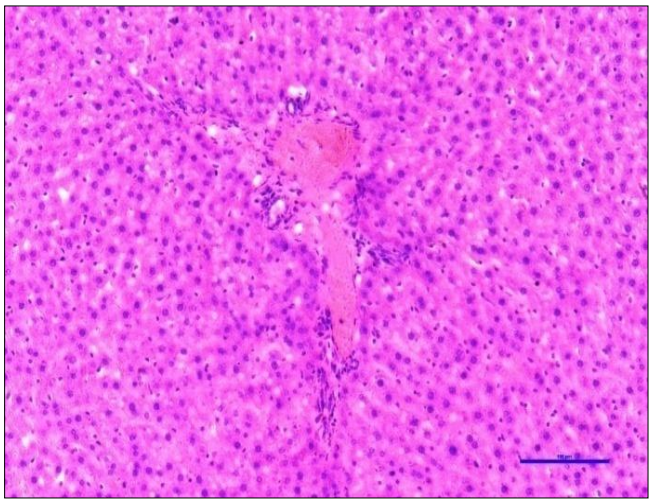

Fig 14: Photomicrograph of liver section revealing disorganization of hepatocytes with Neutrophilic infiltration around the congested central vein of liver

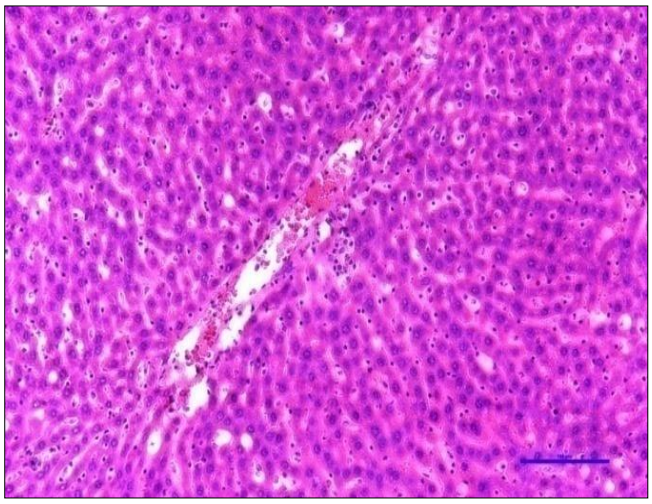

Fig 15: Photomicrograph of liver section revealing degeneration of the hepatocytes, dilated blood vessel with red blood cells and Neutrophilic cellular infiltration

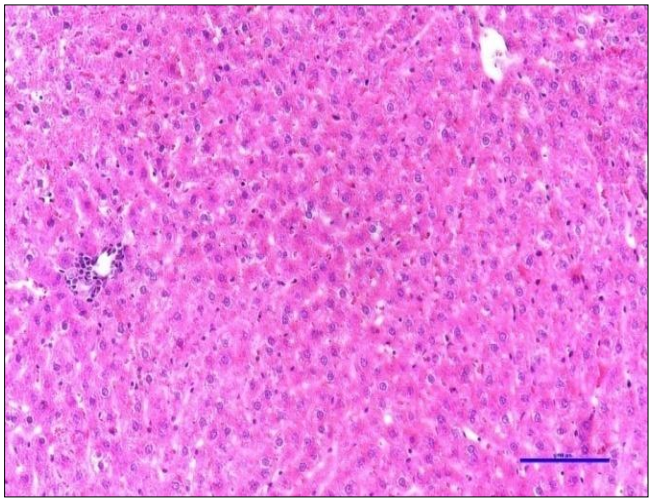

Fig 16: Photomicrograph of liver section revealing mild Perivascular cellular infiltration with degeneration of the hepatocytes

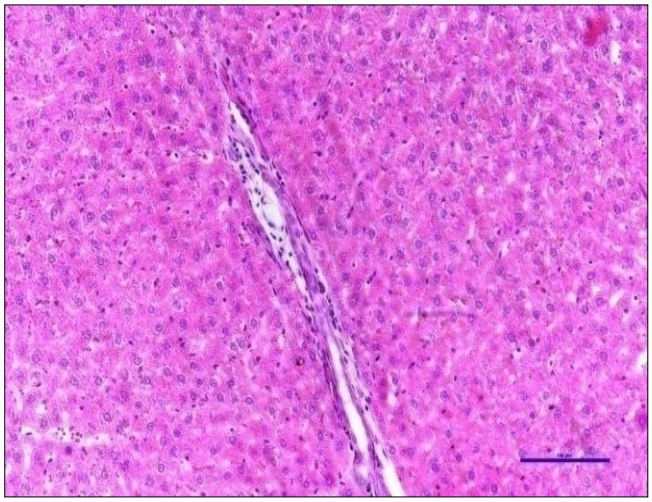

Fig 17: Photomicrograph of liver section revealing necrosis of hepatic sinusoids with cellular infiltration

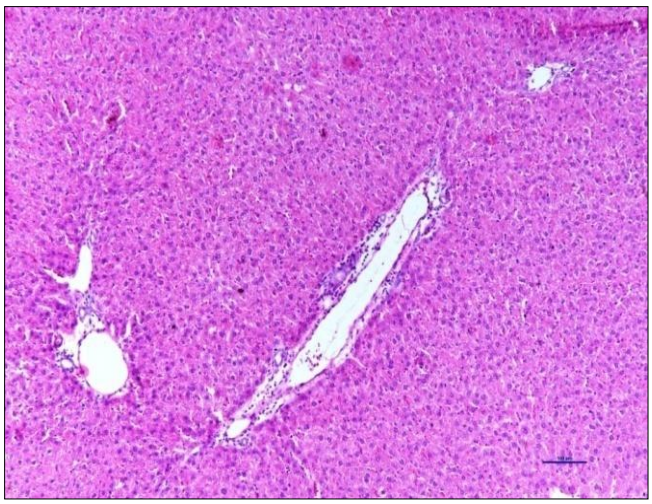

Fig 18: Photomicrograph of liver section revealing sinusoidal degeneration with cellular infiltration around the blood vessel

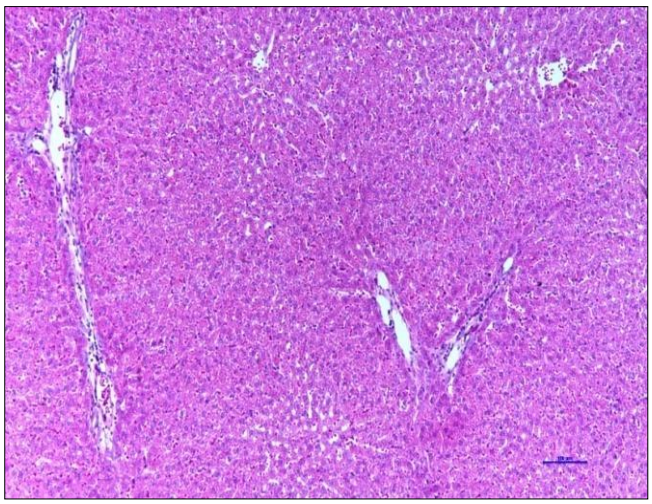

Fig 19: Photomicrograph of liver section revealing hepatocellular degeneration with Perivascular neutrophilic infiltration

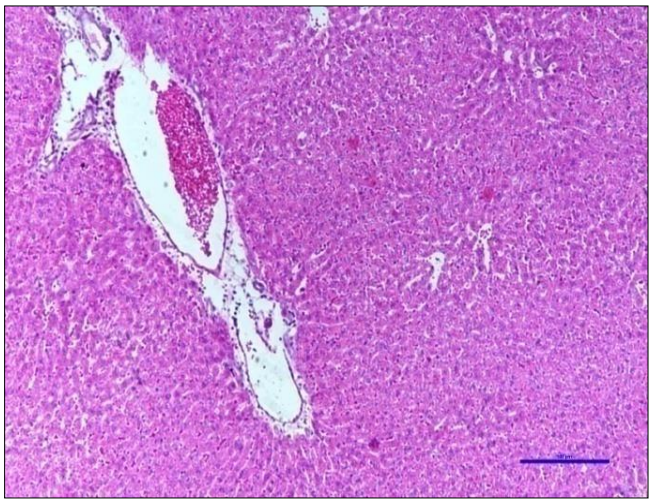

Fig 20: Photomicrograph of liver section revealing congested central vein with Necrosis of hepatocytes

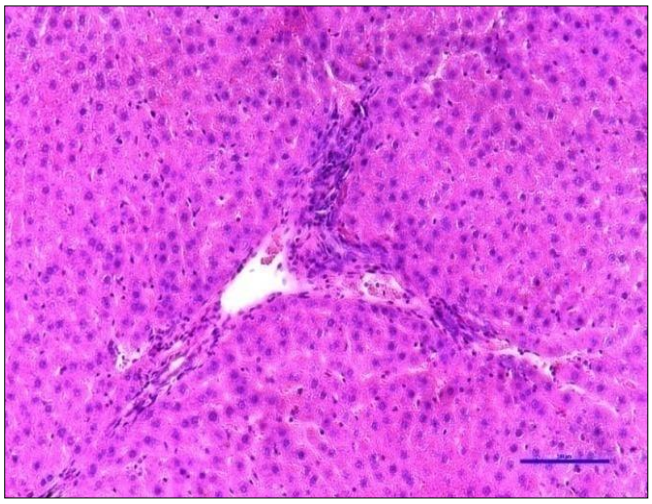

Fig 21: Photomicrograph of liver section revealing mild congestion with marked cellular infiltration

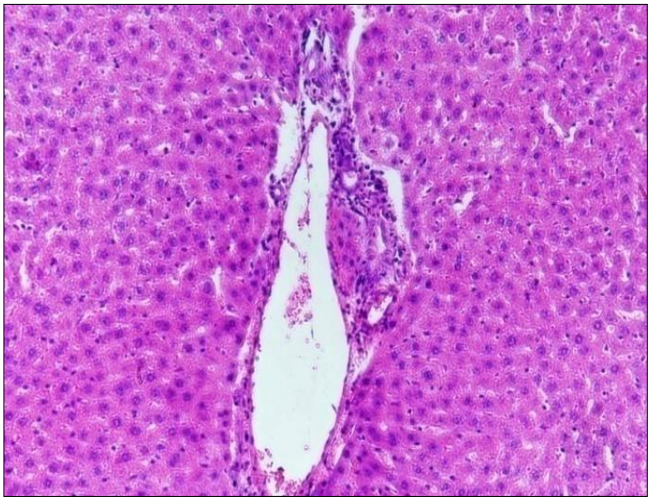

Fig 22: Photomicrograph of liver revealing narrow hepatic sinusoids lined by endothelial cells and cellular infiltration around wall of the blood vessel

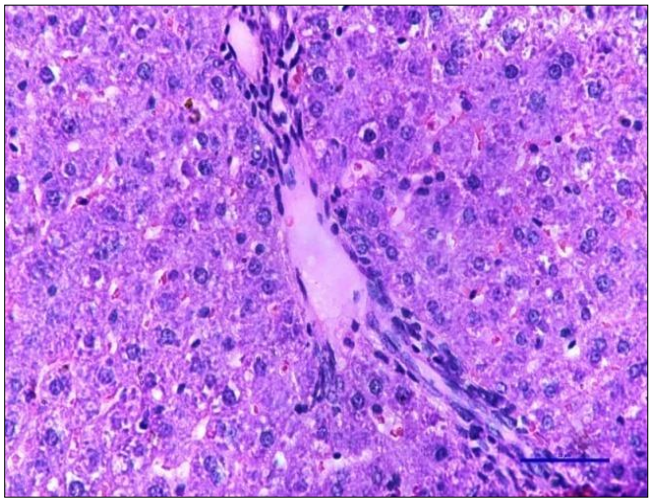

Fig 23: Photomicrograph of liver revealing mild congestion, hepatocellular necrosis and Neutrophilic infiltration

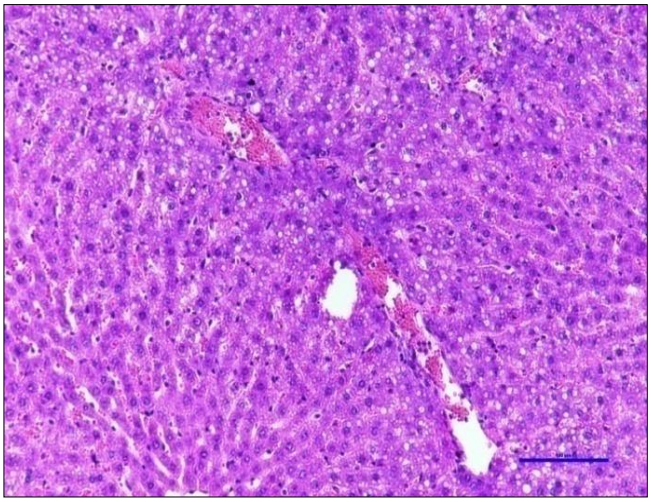

Fig 24: Photomicrograph of liver showing Lumen of blood vessel engorged with Red blood cells, necrosis of hepatocytes and degeneration

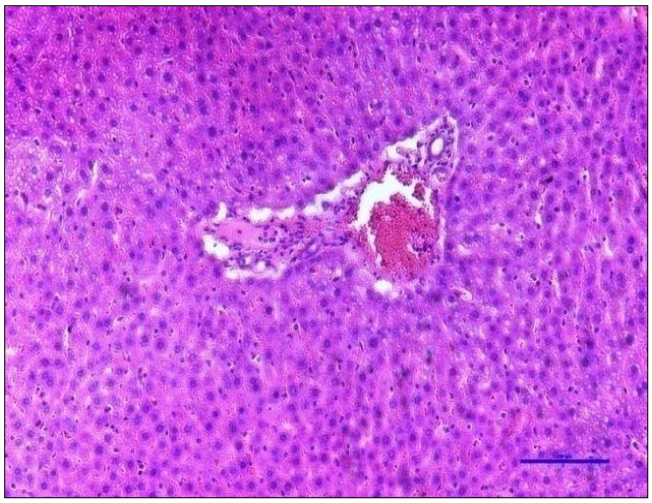

Fig 25: Photomicrograph of liver revealing congested central vein, necrosis and Perivascular cellular infiltration

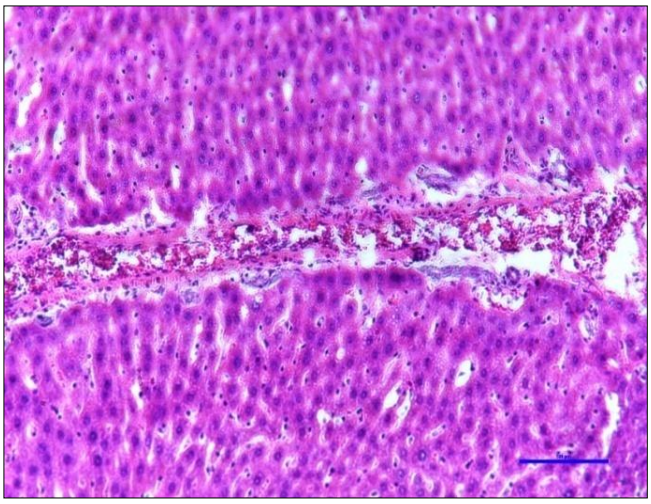

Fig 26: Photomicrograph of liver revealing individualization of hepatocytes and necrosis

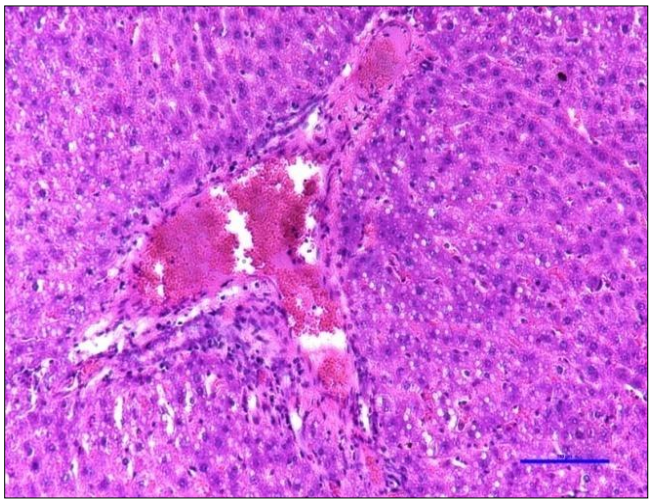

Fig 27: Photomicrograph of liver revealing engorgement of blood vessel with severe cellular infiltration

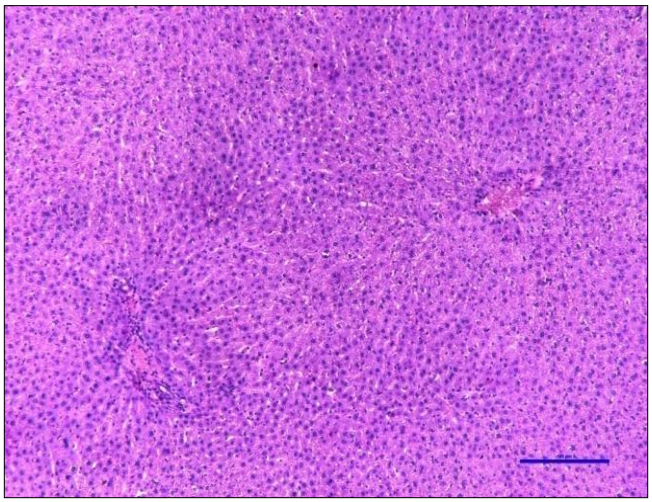

Fig 28: Photomicrograph of liver revealing engorged central vein with blood cells, degeneration and severe cellular infiltration

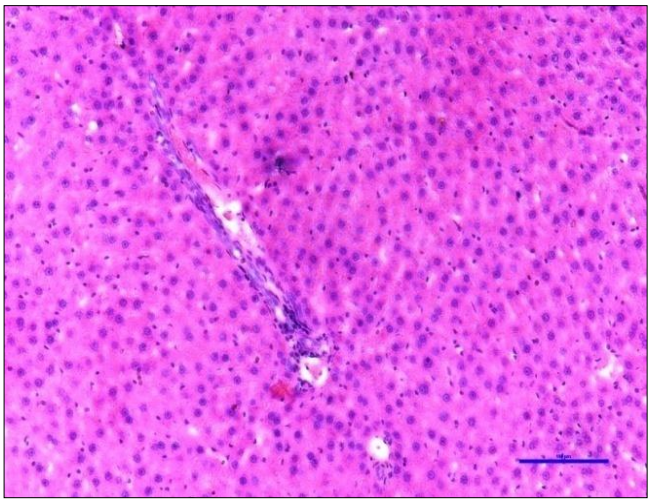

Fig 29: Photomicrograph of liver revealing indivilisation of hepatocytes with severe cellular infiltration on the wall of blood vessel

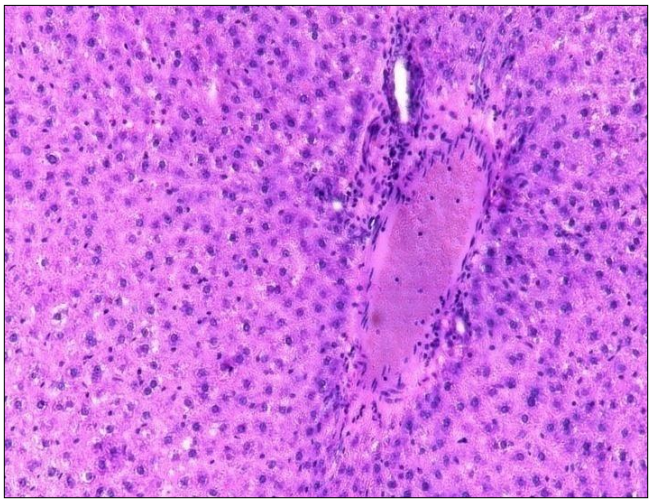

Fig 30: Photomicrograph of liver revealing sinusoidal necrosis with severe perivascular cellular infiltration

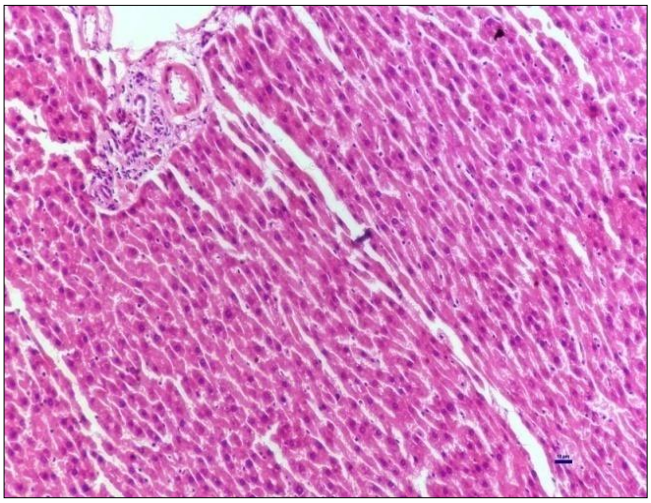

Fig 31: Photomicrograph of liver revealing thickening of the wall of the blood vessel with Neutrophilic cellular infiltration

Relative Organ Weight

The statistical analysis of relative organ weight index at the end of experimental period revealed significant increase in case of metformin intoxicated rats when compared with control group as shown in table.5

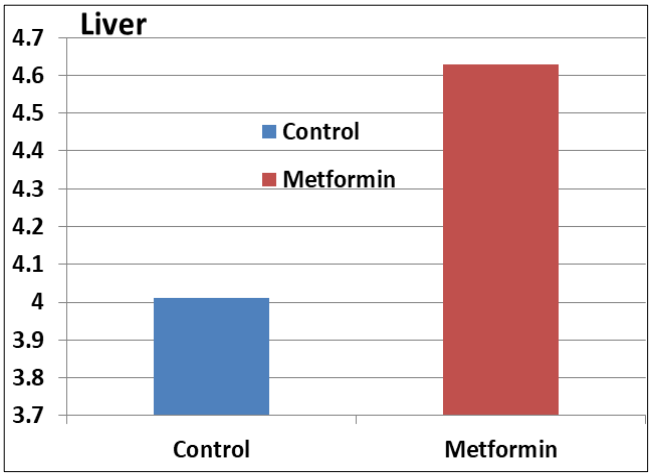

Fig 32a: Alteration in relative organ weight of liver in metformin intoxicated rats

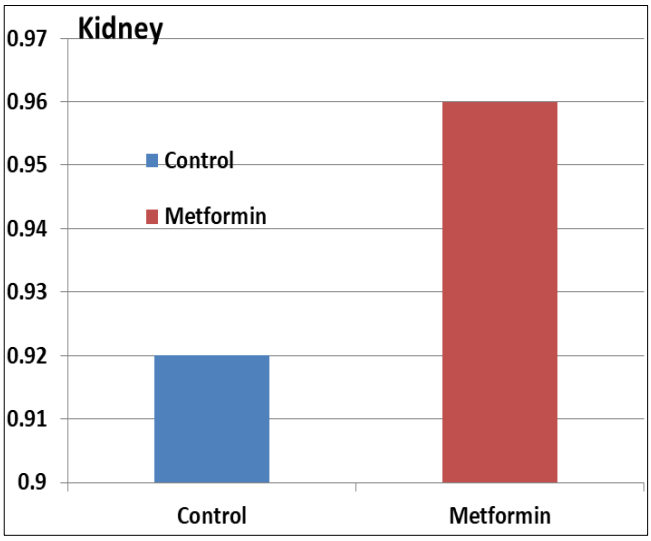

Fig 32b: Alteration in relative organ weight of kidney in metformin intoxicated rats

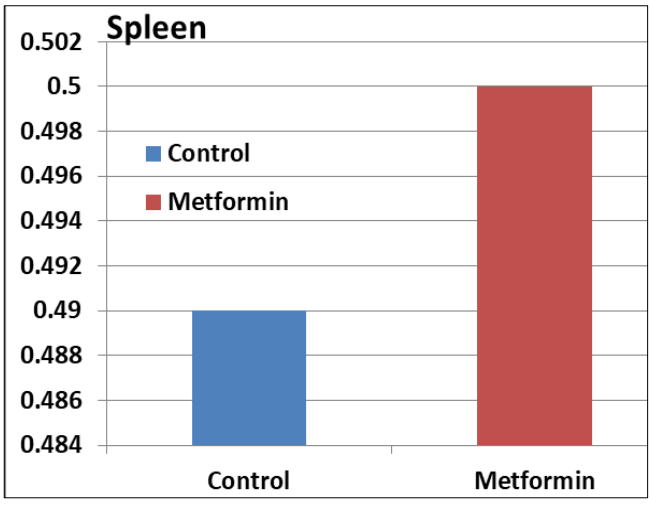

Fig 32c: Alteration in relative organ weight of spleen in metformin intoxicated rats

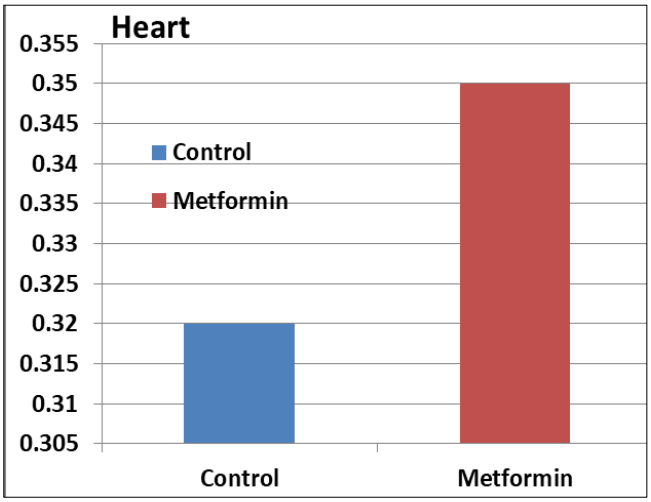

Fig 32d: Alteration in relative organ weight of heart in metformin intoxicated rats

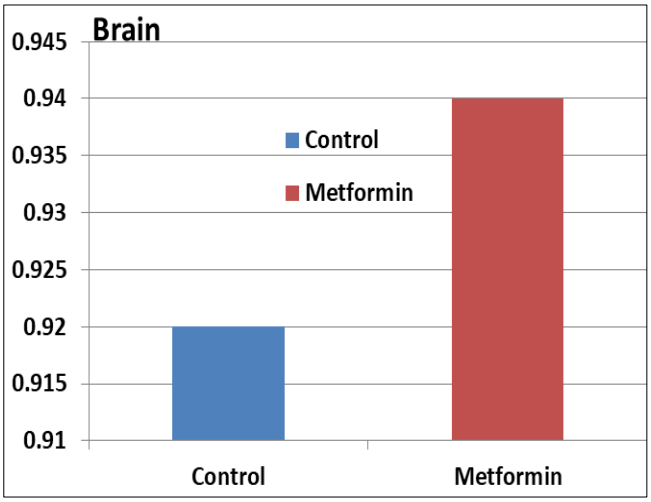

Fig 32e: Alteration in relative organ weight of brain in metformin intoxicated rats

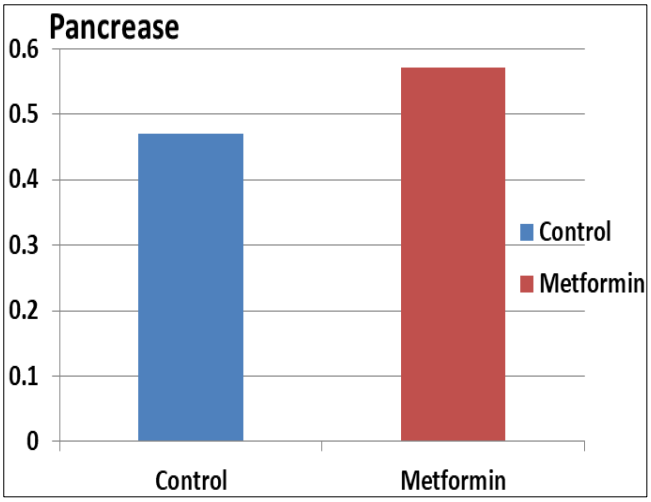

Fig 32f: Alteration in relative organ weight of Pancreases in metformin intoxicated rats

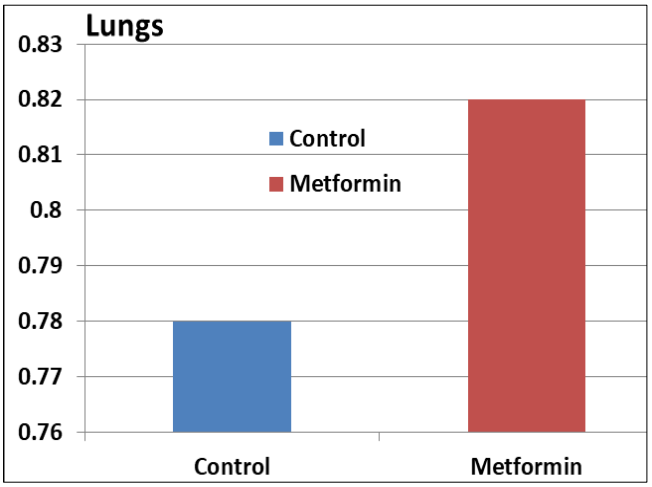

Fig 32g: Alteration in relative organ weight of Lungs in metformin intoxicated rats

Table 5: Mean ± S.D. on Relative Organ Weight (ROW) in Wister rats after intoxicated with Metformin

Relative Organ Weight (g)							
Groups	**Liver**	**Kidney**	**Spleen**	**Heart**	**Brain**	**Pancrease**	**Lungs**
Control	4.01 ^{a}±0.24	0.92 ^{a}±0.08	0.49 ^{a}±0.06	0.32 ^{a}±0.02	0.92 ^{a}±0.06	0.47 ^{a}±0.08	0.78 ^{a}±0.02
Metformin	4.63 $^{b\pm}$0.25	0.96 ^{b}±0.17	0.50 ^{b}±0.03	0.35 ^{b}±0.04	0.94 ^{b}±0.08	0.57 ^{b}±0.09	0.82 ^{b}±0.04

Means of Relative Organ Weight differ significantly between the groups

Kidneys

During the study period, scarification of the animals revealed larger, swollen kidneys with petechiael hemorrhages on their surface. The kidneys throughout this investigation appeared normal on day 5, but by day 21 they were mottled with a grayish color and had necrotic foci on their surface.

Histopathology of control group

The renal cortex in the kidney sections of control rats appeared normal upon inspection. Normality the Two layers of epithelium and Bowman's capsule surround the glomerulus. The proximal convoluted tubules were bordered with simple epithelia, and the glomeruli are rounded. These epithelia possessed an acidophilic cytoplasm and an abundance of microvilli at the apex, which together created a brush-like border. The simple cuboidal epithelium lined the distal convoluted tubules.

Histopathology of Metformin treatment group

When the metformin-treated animal was sacrificed on day 7, it showed cellular infiltration-driven interstitial nephritis, necrotic alterations in the renal tubules, and hepatocellular deterioration. The glomeruli showed that the parietal and visceral glomerulus layers had been destroyed, increasing Bowman's space and coagulative necrosis. In addition, there was glomerular atrophy, which reduced the prominence of the glomerular structure.

The proximal and distal convoluted tubules of the affected animals revealed displayed desquamated cells, pyknotic nuclei, and denuded renal epithelium. The destructed renal tubules, uneven mesangial regions, a loss of glomerular characteristic look, and intratubular hemorrhages were all visible in the kidney sections of the affected rats when they were sacrificed at day 14. The damaged rats' kidneys raveled disordered glomeruli, a widening of Bowman's space, most of the renal tubules were dilated, destroyed, and the epithelial cells were degenerating by the end of the study period.

Histopathology

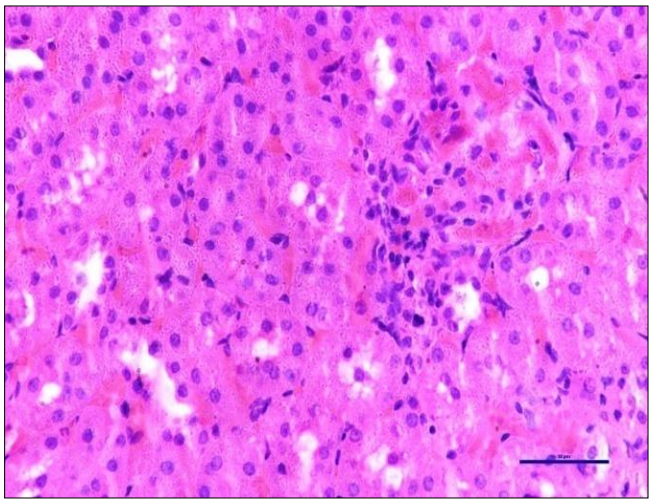

Fig 33: Photomicrograph revealing sloughing of renal tubular epithelium with Nephritis

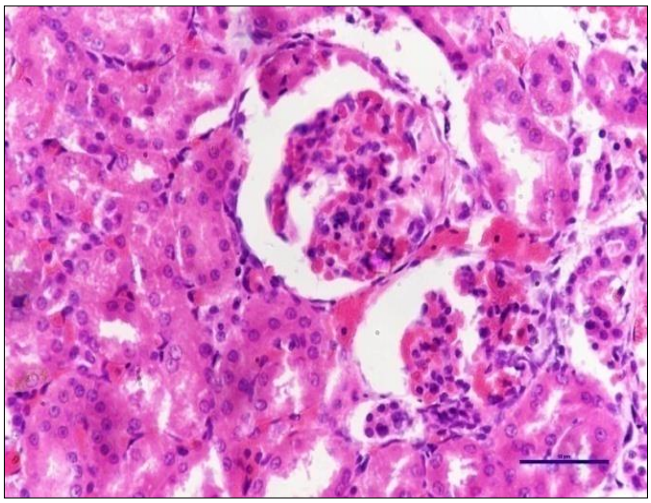

Fig 34: Photomicrograph revealing glomerular atrophy and destruction of parietal and visceral layer of glomerulus

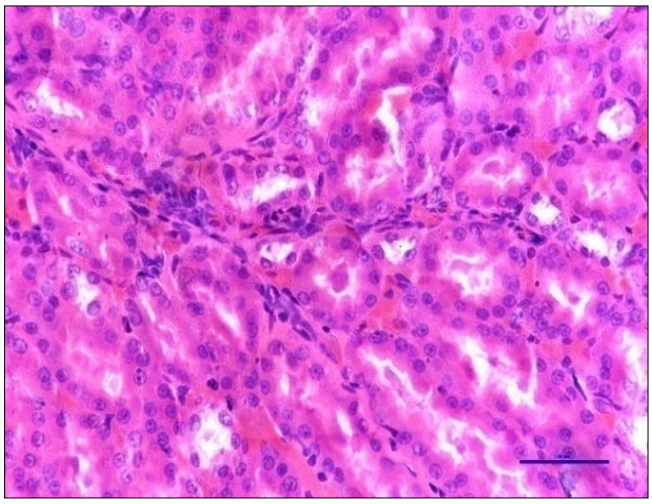

Fig 35: Photomicrograph revealing degeneration of renal tubular epithelium, congestion and severe cellular infiltration in the renal tissue

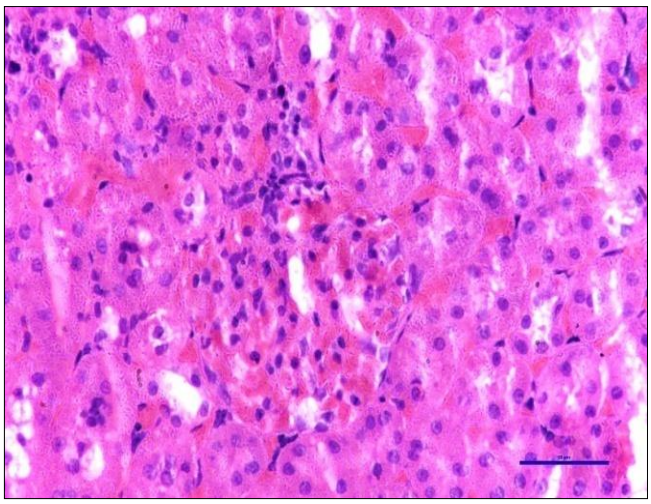

Fig 36: Photomicrograph revealing nephritis as evident by severe infiltration of inflammatory cells along with congestion in the renal tissue

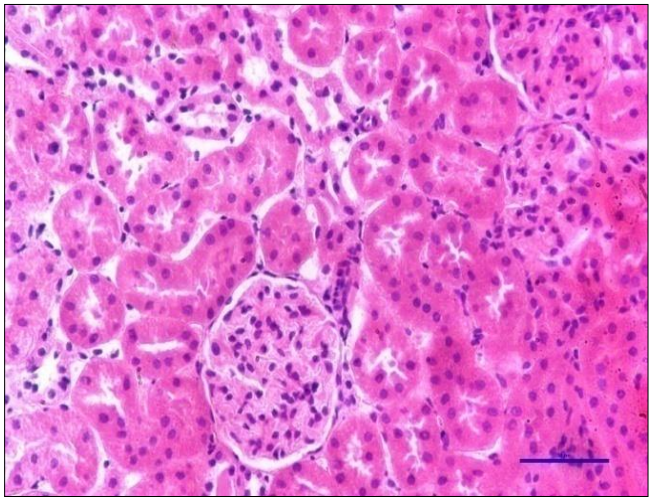

Fig 37: Photomicrograph revealing interstitial nephritis in association with renal tubular degeneration

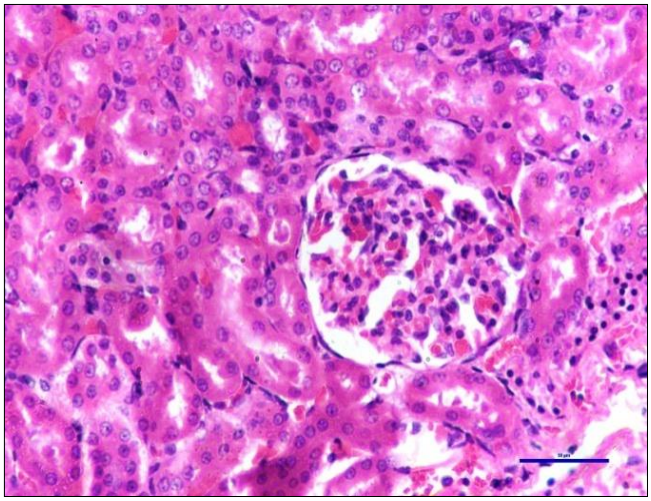

Fig 38: Photomicrograph revealing severe necrosis with sloughing of renal tubular epithelium

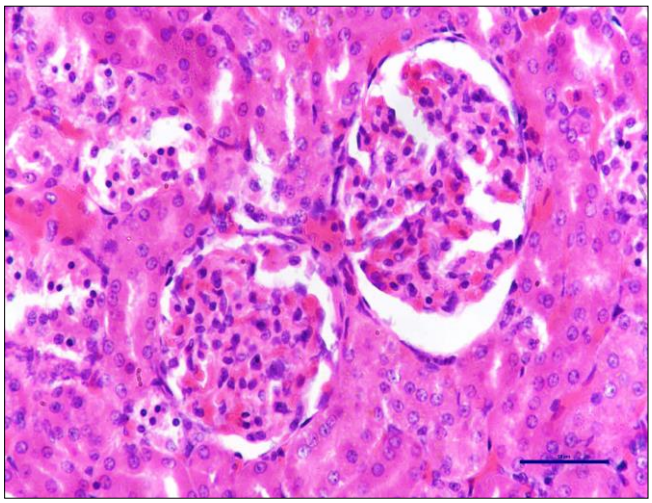

Fig 39: Photomicrograph revealing glomerular atrophy along with renal tubular degeneration

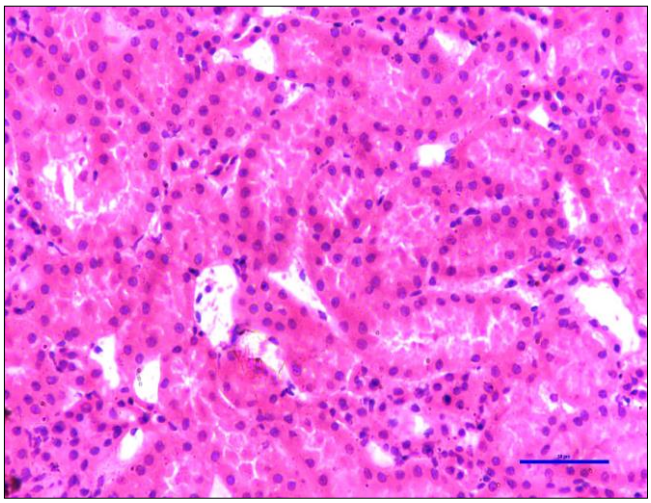

Fig 40: Photomicrograph revealing enlarged renal tubulules, necrosis with severe cellular infiltration in the intersitium

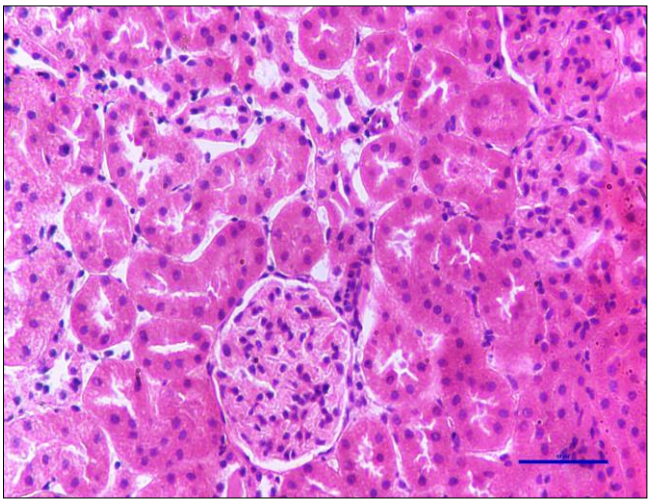

Fig 41: Photomicrograph revealing glomerular and interstitial nephritis in association with sloughing of renal tubular epithelium

Discussion

When the pancreas is unable to produce insulin, diabetes develops as a chronic condition. The pancreas produces the hormone insulin, which functions as a key to allow glucose from the bloodstream to enter into the body's cells where it may be used to make energy. Animals with prediabetes and diabetes are frequently treated with metformin as reported early by Ortega *et al*., 2014) [18]. Metformin lowers blood sugar levels by reducing gluconeogenesis, also known as hepatic glucose synthesis, intestinal glucose absorption, and insulin sensitivity by raising peripheral glucose uptake and utilization. According to Chen *et al*. (2011) [5], diabetic people are more susceptible to a number of problems, including nephropathy, retinopathy, and neuropathy.

According to Rafieian *et al*. (2013) [23], chlorpropamide treatment and metformin have different effects on controlling diabetes. According to a prior study by Kim *et al*. (2015) [10], metformin has the ability to lower the frequency of angina, stroke, and sudden death in diabetic individuals. Across time, people around the world have come to know that these anti-diabetic medications could be hazardous to both people and animals. Our ultimate objective should be to understand the toxicipathological impact of metformin on the liver and kidneys of humans and other animals because the danger posed by this medication lies in their persistent effects.

Twenty Wistar rats were randomly divided into two groups for this investigation, with ten rats kept in each group. In this study, the first group acted as the control, while the second group took metformin hydrochloride orally once day at a dose of 30 mg/kg body weight for a total of 21 days. The dose rate was chosen based on prior research, as reported early by Oluwatosin *et al*. (2012) [19]. This study unequivocally shown that metformin administration over an extended period of time did not result in any appreciable alterations to the hematological markers. These results corroborated those of other investigations, including those by Muhammad *et al*. (2012) [14], Charity *et al*. 2019 [6], and Fagbohun *et al*. 2020 [7]. This finding could be correlated to the similar results reported earlier by Soheir *et al*., (2011) [26] after investigating the effect of metformin in rats which resulted in the increased levels of Aspartate Transaminase. Nathan *et al*., (2008) [16] also reported increased level of Aspartate Transaminase in metformin intoxicated rats. This increase might be due to the hepatic potency of metformin resulting in destructive changes in the hepatic cells of rats. Metformin caused significant increase in the activity of Alkaline Phosphatase enzyme in all the intoxicated rats. This finding could be correlated to the similar results reported earlier by Nathan, *et al*. (2008) [16]. This elevation in the level of serum alkaline phosphatase in the present study might be due to hepatotoxic effect of the drug as observed by Soheir *et al*., (2011) [26].

In the toxicity group, our investigation found that metformin significantly increased the activity of the enzyme aspartate transaminase (AST). The results of Soheir *et al*. (2011) [26] 's investigation into the effects of metformin in rats, which led to elevated levels of aspartate transaminase, and this finding may be related. In rats given metformin intoxication, Nathan *et al*. (2008) [16] also noted an elevated level of Aspartate Transaminase. This rise may be caused by the hepatic potency of metformin, which causes harmful alterations in the rat liver cells. In all the drunk rats, metformin significantly increased the activity of the enzyme alkaline phosphatase**.** This elevation in the level of serum alkaline phosphatase in the present study might be due to hepatotoxic effect of the drug as observed by Soheir *et al*., (2011) [26].

When compared to the control group, a substantial change in the level of blood total protein was seen in the affected rats. This discovery might be related to the findings presented by Khadre *et al*. (2011) [11]. The pathomarphological abnormalities in the liver and kidneys seen in this study may be the cause of the shift in serum total protein levels. All metformin-intoxicated rats showed a considerable rise in blood albumin levels, which is consistent with earlier research by Viswanathan *et al*. (2004) [27]. This rise in albumin indicates that the kidneys are unable to reabsorb the protein they make through the renal tubules as is typically the case. The elevated albumin level in the serum of intoxicated rats clearly demonstrated that continuous drug use may cause significant renal impairment. The pathomorphological observations of kidneys, as previously described by Kroustrup *et al*., (1997) [9], provide complete support for this claim.

The Rats given metformin were found to have higher blood albumin levels. Early renal insufficiency and early renal injury may be to blame for this rise in serum albumin levels. Serum albumin levels are higher when there is a larger concentration of albumin. This discovery might be related to the comparable outcomes previously reported by Viswanathan *et al*. (2004) [27]. According to Lerma *et al*. (2009) [12], renal

injury may be the cause of the increase in albumin level. All of the drunk rats in this investigation had significantly higher blood globulin levels than control rats. This finding could be correlated with the results reported earlier by Khadre *et al*., (2011) [11] in rats after the exposure with metformin. The reason might be that metformin causes pathomorpholoical changes in the liver which is responsible for the alternation in the serum globulin level in the intoxicated rats.

When comparing the blood cholesterol and triglyceride levels of all metformin-impaired rats to the control group at the end of the study period, the current study found a substantial drop in both. These results are consistent with those obtained in rats by Mishra *et al*. (2019) [15]. Hepatopathy, which lowers the level of serum cholesterol and triglycerides, may be the cause of hypertriglyceridemia and hypocholesterolemia in the inebriated rats.

In comparison to the control group, the experimental animals' serum levels of LDL, HDL, and VLDL were lower after receiving metformin. This lipids profile was consistent with earlier research by Anurag and Anuradha (2002) [1] that examined lipid levels after administering metformin to rats. This drop in lipid profile could be brought about by metformin's damaging effects on rats' kidneys, which result in altered renal function. Intoxicated rats' lipid profiles were directly impacted by metformin, according to Patel *et al*.'s 2019 [20] paper. The findings first reported in rats by Obi *et al*. (2016) [17] and the falling trend of serum lipid profile are both connected.

Conclusion

From this study, it was concluded that the Metformin intoxicated rats revealed an increase in liver function test when compared with control group. Furthermore, the antioxidants biomarkers were decreased accompanied with alterations in histological architectures of liver and kidney tissues. Therefore, it is suggested that the prolonged treatment of metformin revealed a hepatorenal dysfunctions in rats.

Conflict of interest: Authors declare that there was no conflict of interest.

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1. Anurag P, Anuradha V. Metformin improves lipid metabolism and attenuates lipid peroxidation in high fructose-fed rats. Diabetes Obesity Metabolism. 2002;24(1):36-42.
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