

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(1): 518-525
www.biochemjournal.com
Received: 01-10-2025
Accepted: 05-11-2025

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Protective effects of garlic-derived lectin on carbon tetrachloride-induced hepatotoxicity in male wistar rats: Impacts on gross pathology and histopathology

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i1g.7007>

Abstract

Background: Chemical-induced liver injury remains a major preclinical model for evaluating hepatoprotective agents. This study examines whether a garlic-derived lectin can ameliorate hepatic damage in rats with Carbon tetrachloride (CCl₄)-induced hepatotoxicity.

Methods: Thirty-six age-matched rats were randomly allocated into six groups (n = 6 each): (1) normal control; (2) disease control (CCl₄ only); (3) reference control receiving Silymarin (200 mg/kg, orally); and (4-6) treatment groups receiving garlic lectin intraperitoneally at 10, 20, or 40 mg/kg twice weekly. Hepatotoxicity was induced by IP injection of CCl₄ (2 mL/kg in 50% vegetable oil) twice weekly for five weeks; lectin or silymarin treatment was administered from weeks 6 to 8. At the end of treatment, liver tissues were collected for histopathological evaluation.

Results: Histopathological assessment revealed that CCl₄ administration produced characteristic hepatic lesions, including microvesicular and macrovesicular steatosis, bile-duct proliferation, single-cell necrosis, and centrilobular necrosis. Treatment with garlic-derived lectin at 20 mg/kg and 40 mg/kg resulted in marked attenuation of these lesions: the prevalence and severity of steatosis, bile-duct proliferation, and necrotic foci were substantially reduced compared with the disease control. The extent of histological recovery in these groups approached that observed in the silymarin-treated reference group.

Conclusion: Garlic-derived lectin at doses of 20 and 40 mg/kg confers significant hepatoprotection in CCl₄-induced liver injury in rats, as demonstrated by pronounced improvement in liver histopathology. These findings support further investigation of garlic lectin as a promising candidate for mitigating chemically induced hepatic damage.

Keywords: Garlic-derived lectin, carbon tetrachloride, hepatotoxicity, rat liver, histopathology, hepatoprotection

Introduction

Drug Induced Liver Injury (DILI) includes the whole spectrum from asymptomatic and transient elevation in liver tests to acute liver failure (ALF). Drug induced Liver injury due to paracetamol overdose and idiosyncratic drug reactions remain the most common causes of ALF in the Western world. Despite recent research showing a large rise in idiosyncratic DILI caused by herbal and nutritional supplements, antimicrobials continue to be the most common cause of DILI. Comparing cholestatic or mixed DILI to hepatocellular DILI, ALF progression is more common (Katarey and Verma, 2016) [1].

Brautbar and Williams (2002) [2] opined that Carbon tetrachloride (CCl₄) is one of the best known hepatic toxins used in preclinical models to investigate the hepatoprotective effect of various medicinal plants and natural products. They also reported that severe liver necrosis, as well as steatosis can be induced by a direct single exposure to CCl₄ as it is a powerful hepatotoxic and xenobiotic agent.

Non-alcoholic fatty liver disease (NAFLD) includes a range of histological liver conditions, from isolated steatosis to steatohepatitis and cirrhosis, that are linked to obesity, diabetes, and insulin resistance. Pathogenic theories for NAFLD/NASH must take into account the close ties between excessive dietary intake and inactivity, insulin resistance and hereditary variables.

Lipotoxicity, oxidative stress, cytokines, and other pro-inflammatory mediators may each play a role in transition of steatosis to Non-alcoholic steato hepatitis (Adams *et al.*, 2005) [3].

Sharon and Lis (2004) [4] reported that Lectins' recognition of cell-surface carbohydrates has a wide range of biological ramifications. Lectins have become a paradigm for protein-carbohydrate recognition due to their capacity to detect minor differences in carbohydrate structures on the surface of cells and tissues.

Lectins are typical globular proteins of nonimmune origin displaying binding to specific carbohydrates of oligo/polysaccharides, glycoproteins and glycolipids. Among the many biological effects of dietary lectins, their immunomodulatory effects have been well studied (Van Damme *et al.*, 2008) [5].

Lai *et al.* (2014) [6] investigated the protective properties of garlic essential oil and its major organosulfur component (diallyl disulfide, DADS) against the development of nonalcoholic fatty liver disease (NAFLD). DADS significantly decreased the release of pro-inflammatory cytokines in liver, accompanied by elevated antioxidant capacity *via* inhibition of cytochrome P450 2E1 expression during NAFLD development.

Materials and Methods

The present work "Studies on effects of Garlic lectin in Carbon tetrachloride induced hepatotoxicity in male wistar rats" was carried out during academic year 2021-22 at the department of Veterinary Pathology, Veterinary College, Hassan, KVAFSU, Bidar. The study was conducted on adult healthy male Wistar albino rats. Healthy adult male Wistar rats weighing 180-200g. To induce experimental hepatotoxicity in rats, CCl₄ was procured from Sigma Aldrich Corporation, St. Louis, USA with molecular weight 153.82; CAS number 56-23-5. All other chemicals and reagents used for the study were of analytical grade. It was injected intraperitoneally at the rate of 2ml/kg body weight 50 percent in vegetable oil twice a week for five weeks (Khan *et al.*, 2012, Elsayy *et al.*, 2019) [11, 12]. Garlic derived lectin extracted purified and characterized was procured from Department of Biochemistry, Davangere University, Shivangotri, Davangere. The working injectable Garlic lectin solution was prepared in fresh phosphate buffered saline having a pH of 7.4 and the same was maintained at 4-8 °C. The required quantity of garlic lectin was dissolved in ice-cold phosphate buffered saline and injected intraperitoneally to rats immediately to avoid degradation. Silymarin available commercially as oral hepatoprotective tablets (silybon®-70) 70 mg tablet manufactured by Micro lab ltd), was purchased and administered orally at the dose rate of 200mg/kg bw/weekly four times for three weeks from six to eight weeks of study (Tsai *et al.*, 2008) [7].

Experimental protocol:

Rats were selected randomly and divided into six groups (Group I to VI) each consisting of six rats. Group I served as normal control group and Group II served as CCl₄ disease control. All the rats were numbered group wise and individually. Hepatotoxicity was induced in Group II to VI animals using Carbon tetrachloride (50 % in Vegetable oil) @ 2 ml/kg bw twice a week for first five weeks. The effects of CCl₄ treatment and induction of hepatotoxicity was studied in the rats of Group II that served as CCl₄ disease control.

Parameters assessed

Gross pathology liver and other vital organs were examined immediately after sacrifice on the final day of the experiment for colour changes, fragility, size, consistency and lesions produced in the due course of experimental period and recorded and Histopathology.

Statistical analysis

Statistical analysis of the data collected for various parameters was done using one-way ANOVA and Duncan's multiple-range test (Hamada, 2018) [8] using the software (SPSS, version-16.0)

Results

Gross Pathology

The liver and other vital organs of normal control rats (Group I) were examined immediately after sacrifice on the final day of the experiment for colour changes, fragility, size, consistency and lesions produced in the due course of experimental period and recorded. Liver appeared apparently normal with no visible gross pathological changes in its architecture and size (Fig.1).

The livers of the disease control rats (Group II) appeared mild to moderately enlarged and no lustre, rough and hard consistency, rounded border, focal raised necrotic spots and yellowish discoloration of parenchyma (Fig. 2). The other vital organs such as spleen, kidney, brain, heart, intestines, spleen and lungs appeared normal with no detectable gross abnormalities.

The livers of the rats in Group III to VI had minimal to milder changes compared to control rats (Group II). The livers were less swollen with normal lustre. Mild improvement was seen in comparison to disease control group (Fig. 3-6). The vital organs spleen, kidney, heart and lungs appeared normal with no detectable gross abnormalities.

Histopathology and severity scores of major HP lesions:

The mean HP severity score values with standard error of mean have been presented in Table1.

Histopathology lesions of Group I

The liver of control rats (Group I) showed normal liver histological picture with unaffected hepatic cells with well preserved cytoplasm and nucleus. Each hepatic lobule was made up of radiating plate strands of cells forming a network around the central vein with narrow sinusoids (Fig. 7 and 8).

Liver of the Group I normal control rats did not reveal macrovesicular steatosis, kupffer cell hyperplasia, fatty cyst formation, centrilobular necrosis, hepatocyte swelling, fibrosis and bile duct proliferation. Hence, the histopathology score of zero was considered to these microscopic lesions. However for minimal focal microvesicular steatosis and single cell necrosis with scoring 0.33±0.12 and 0.23±0.14, respectively was observed.

Histopathology lesions in Group II (DC)

The livers of disease control group (Group II) showed profound histopathological changes characterized by distorted architecture, dissociation of hepatocytes, sinusoidal dilation and mild to moderate cellular swelling. Hepatic lobules also revealed severe degenerating

hepatocytes with granular and basophilic cytoplasm, necrotic changes with pyknotic and fragmentation of nucleus. The pattern of distribution of necrosis was predominantly multifocal centrilobular (Fig. 9), Multiple areas of moderate to severe macrovesicular steatosis, with hepatocytes containing a large and single vacuole of fat peripherally displaced nucleus, giving rise to a characteristic signet ring appearance in some areas was observed (Fig. 10). These macrovesicular vacuoles were fused to form fatty cyst with amorphous eosinophilic material in few areas. Moderate to severe bile duct proliferation (Fig.11), moderate to severe kupffer cell hyperplasia was seen. Single cell necrosis was severe in all the lobules making an island/pseudolobule and few hepatocytes have become apoptotic adjacent to necrotic area with some multifocal mononuclear cell infiltration.

Fat staining by osmium tetroxide method confirmed the presence of lipid material (Fig. 12 and 13). Minimal to mild proliferation interlobular connective tissue and moderate to severe of peri-hepatic fibrous capsule and mesothelial cells proliferation beneath it was recorded, Masson's Trichrome staining confirmed this connective tissue proliferation (Fig. 14).

The mean HP severity scores of liver lesion of Group II disease control rats were 2.83 ± 0.3 , 4.08 ± 0.2 , 3.5 ± 0.22 , 4.16 ± 0.30 , 3.33 ± 0.42 , 4.1 ± 0.25 , 3.3 ± 0.42 , 3.16 ± 0.3 and 2.25 ± 0.17 for hepatocyte swelling, microvesicular steatosis, macrovesicular steatosis, fatty cyst formation, kupffer cell hyperplasia, single cell necrosis, centrilobular necrosis, bile duct proliferation and fibrosis, respectively. The mean HP severity score of Group II disease control rats for each of the recorded lesion was statistically significant ($p < 0.05$) than the mean HP score of Group I normal control rats.

Histopathology lesions in Group III (RC)

The histopathological lesions in the liver of Group III rats treated with silymarin revealed minimal hepatocyte swelling, mild microvesicular steatosis and minimal to mild macrovesicular steatosis with minimal to mild fatty cyst formation with amorphous eosinophilic material in it (Fig 15), minimal to moderate kupffer cell hyperplasia and minimal to mild bile duct proliferation, minimal to mild single cell necrosis in some lobes of liver and mild centrilobular degeneration with minimal fibrosis of liver capsule. Mild single cell necrosis in lobule. It appeared that there was a good amelioration of in liver lesions when compared to disease control (Group II). These lesions were minimal to mild compared to disease control (Group II) rats. The mean HP severity score of Group III rats for hepatocyte swelling microvesicular steatosis, macrovesicular steatosis, fatty cyst formation, kupffer cell hyperplasia, single cell necrosis, centrilobular necrosis, bile duct proliferation and fibrosis were 0.66 ± 0.21 , 1.91 ± 0.2 , 1.16 ± 0.16 , 1.33 ± 0.16 , 1.45 ± 0.1 , 1.16 ± 0.1 , 1.91 ± 0.3 , 1.66 ± 0.33 and 0.83 ± 0.16 , respectively. For each of the recorded parameters, the mean HP severity score of Group III rats was significantly ($p < 0.05$) higher than the mean value of HP scores of normal control rats indicating that the CCl₄ induced hepatotoxicity lesions had not resolved completely. However, HP severity score of Group III were significantly ($p < 0.05$) lesser when compared to disease control group.

Histopathology lesions in Group IV to VI

The livers of Group IV rats treated with garlic lectin @ 10 mg/kg revealed histopathological lesions that were lesser in

severity compared to those in the livers of rats in disease control group. The lesions consisted of minimal hepatocyte swelling and mild to moderate microvesicular steatosis with mild macrovesicular steatosis, mild fatty cyst formation. Also mild to moderate single cell necrotic area showing island of degenerating cells with mild centrilobular degeneration of hepatocytes, mild to moderate kupffer cells hyperplasia (Fig. 16) and mild focal bile duct proliferation with mild fibrosis of liver capsule.

The rats in Group V and VI treated with garlic lectin @ 20 mg/kg and 40 mg/kg, respectively showed similar liver histopathological lesions as that of the rats of Group III control rats. The lesions consisted of minimal hepatocyte swelling and minimal to mild microvesicular steatosis with minimal macrovesicular steatosis and fatty cyst formation (Fig. 17). Also, minimal to mild single cell necrotic area showing island of degenerating cells (Fig.18) with minimal to mild centrilobular degeneration of hepatocytes, minimal to mild kupffer cells hyperplasia and minimal to mild bile duct proliferation with minimal fibrosis of liver capsule.

The mean HP severity score of Group IV rats for hepatocyte swelling, microvesicular steatosis, macrovesicular steatosis, fatty cyst formation, kupffer cell hyperplasia, single cell necrosis, centrilobular necrosis, bile duct proliferation and fibrosis were 1.08 ± 0.27 , 2.66 ± 0.16 , 2.08 ± 0.08 , 1.91 ± 0.15 , 2.5 ± 0.42 , 2.2 ± 0.11 , 2.08 ± 0.15 , 1.91 ± 0.27 and 1.16 ± 0.30 , respectively. Similarly, the HP severity scores of Group V rats was 0.75 ± 0.17 , 1.66 ± 0.2 , 1.41 ± 0.15 , 1.16 ± 0.25 , 1.5 ± 0.23 , 1.33 ± 0.21 , 1.5 ± 0.24 , 1.83 ± 0.16 and 1.0 ± 0.31 , respectively and also the HP severity score of Group VI was 0.83 ± 0.16 , 1.58 ± 0.24 , 1.08 ± 0.2 , 1.01 ± 0.16 , 1.33 ± 0.21 , 1.23 ± 0.24 , 1.41 ± 0.27 , 1.33 ± 0.21 and 0.93 ± 0.27 , respectively.

Discussion

Group I

In the present study, the normal control group (Group I) liver revealed normal liver architecture and also spleen, testes and kidney appeared normal. Few spontaneous lesions like single cell necrosis and microvesicular steatosis with minimal severity in liver and were well within the normal microscopic changes that are commonly seen in this age group (Gad, 2007) [9].

Group II

Histologically, the livers from disease control rats (Group II) revealed a widespread damage of liver parenchyma characterized by remarkable microvesicular steatosis, macrovesicular steatosis, kupffer cell hyperplasia, fatty cyst formation, centrilobular necrosis, hepatocyte swelling, bile duct proliferation and single cell necrosis. Further, mild to moderate fibrosis characterized by fibroblast proliferation and accumulation of collagen fibres was evident though these fibrotic changes were considered as healing process in chronic inflammation, it is also well proven fact that inflammation is a double edged sword as fibrotic changes will do more harm than benefit as it is an irreversible process leading to irritation to neighboring cells leading to harmful effect like permanent scarring and organ malfunction. Similar histopathological findings have been recorded in CCl₄ induced hepatotoxicity model by previous researchers (Almatroodi *et al.*, 2020; Elsayy *et al.*, 2019) [11, 12].

The pathological lesions observed in the CCl₄ induced disease Group is attributed to prolonged CCl₄ administration, metabolism of carbon tetrachloride via Cytochrome-P2E1 to highly reactive free radical primary metabolites like trichloromethyl and trichloromethyl peroxy free radicals, are highly reactive and are capable of covalently binding locally to cellular macromolecules, with preference for fatty acids from membrane phospholipids. The free radicals initiate lipid peroxidation by attacking polyunsaturated fatty acids in membranes, setting off a free radical chain reaction sequence. The lipid peroxidation is known to cause membrane disruption, resulting in the loss of membrane integrity (Recknagel *et al.*, 1989) [13]. CCl₄ reduces the hydrolysis of Triacylglycerols and β -oxidation of fatty acids thus, making more fatty acids available for esterification. Disruption of β -oxidation of fatty acids in the mitochondrial causes microvesicular steatosis, with accumulation of tiny lipid vesicles in the cytoplasm of hepatocytes (Brai *et al.*, 2020) [14].

Kupffer cells produce a variety of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and monocyte chemoattractant protein that provokes the activation of HSCs, which produces extracellular matrix protein, in particular collagen I, and subsequently contribute to hepatic injury (Friedman, 2000) [15].

Hepatic injury results in hepatic stellate cells (HSCs) propagation and revolution into a myofibroblast-like phenotype in a process called activation. Pro-inflammatory cytokines and chemokines stimulate HSCs transformation into myofibroblasts, playing a role in activating the production of ECM and also express α -SMA and procollagen-I and are the main source of collagen I, III, and IV and other fibrosis-deposited matrix proteins (Friedman *et al.*, 2000) [15]. Liver macrophages, including Kupffer cells, generate TGF- β to activate HSCs, which are important regulators of liver fibrosis (Farah *et al.*, 2016) [16].

In the present study Splenic capsular thickening mild to moderate was observed in disease control rats. Similar histopathological findings have been recorded in CCl₄ induced hepatotoxicity model by previous researchers (Sayed *et al.*, 2021) [17]. CCl₄ mediated the overexpression of α -SMA & type-I collagen in spleen. (Sayed *et al.*, 2021) [17].

The histopathology lesions in the livers of Group III rats treated with silymarin after the administration of CCl₄ for five weeks consisted of revealed minimal hepatocyte swelling, mild microvesicular steatosis and minimal to mild macrovesicular steatosis with minimal to mild fatty cyst formation with amorphous eosinophilic material in it, minimal to moderate kupffer cell hyperplasia and minimal to mild bile duct proliferation, minimal to mild single cell necrosis in some lobes of liver and mild centrilobular degeneration with minimal fibrosis of liver capsule. Mild degree of single cell necrosis. These lesions were milder and the HP severity score was significantly lesser when compared to the treatment Group IV as well as disease control Group. However histopathological lesions were significantly higher when compared to normal control Group.

Silymarin alleviated the histopathological lesions caused by CCl₄ to a considerable extent. These findings were in agreement with previous observations (Mahli *et al.*, 2015) [18].

Silymarin inhibited the CCl₄-induced hepatic TNF- α , and IL-1 β expression which indicates reduced activation of Kupffer cells, the resident macrophages in the liver, and less

hepatic immune cell infiltration, respectively (Mahli *et al.*, 2015) [18].

It has antioxidant effects via increase of geneexpression of antioxidant enzymes and a number of the most important protection mechanisms against free radicals damage (Karimi *et al.*, 2011) [19].

These lesions were milder and the HP severity score was significantly lesser when compared to the treatment Group IV as well as disease control Group. However, histopathological lesion score was higher when compared to normal control Group.

Groups IV, V and VI

The rats in Group V and VI treated with garlic lectin @ 20 mg/kg and 40 mg/kg, respectively, showed similar liver histopathological lesions as that of the rats of Group III reference control rats. The lesions like minimal hepatocyte swelling and minimal to mild microvesicular steatosis with minimal macrovesicular steatosis and fatty cyst formation. Also, minimal to mild single cell necrotic area showing island of degenerating cells with minimal to mild centrilobular degeneration of hepatocytes, minimal to mild kupffer cells hyperplasia and minimal to mild bile duct proliferation with minimal fibrosis of liver capsule.

The kidneys of Group IV rats treated with garlic lectin @ 10 mg/kg revealed histopathological lesions that were lesser in severity compared to those in the livers of rats in disease control group. The lesions consisted The lesions minimal hepatocyte swelling and mild to moderate microvesicular steatosis with mild macrovesicular steatosis, mild fatty cyst formation. Also mild to moderate single cell necrotic area showing island of degenerating cells with mild centrilobular degeneration of hepatocytes, mild to moderate kupffer cells hyperplasia and mild bile duct proliferation and mild fibrosis of liver capsule.

In the present investigation, Group V and VI treated with garlic lectin @ 20 mg/kg and 40 mg/kg bw IP route respectively revealed moderate to significant improvement in the microscopical changes in comparison to that of disease control Group. Group IV showed minimal to mild improvement in the histopathological changes.

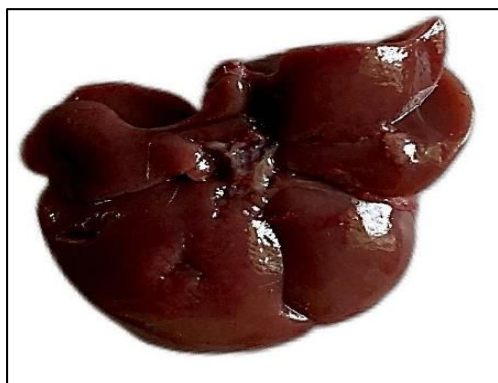
Among the treatment groups V and VI showed significant decrease in HP score values compared to Group IV. Further, no significant variation was observed between Group V, VI and Group III. These data implies that administration of garlic lectin and silymarin brought in considerable protection against histopathological lesions induced by CCl₄.

It has been demonstrated that a different lectin from *Canavalia grandiflora* (ConGF), a D-glucose/D mannose-specific lectin, inhibits the production of a variety of pro-inflammatory cytokines like IL-1 and TNF- α , affecting the leukocyte-endothelium interaction and neutrophil transmigration during inflammation in a dose-dependent manner (Nunes *et al.*, 2009) [20]. Similarly in garlic lectin acted like these leading to anti-inflammatory action leading to reduced necrosis and other inflammatory in liver changes induced by CCl₄.

Liu *et al.* (2002) [21] have recorded that S-alk(en)ylcysteine a derivative of garlic inhibited hepatic cholesterol biosynthesis primarily by decreasing 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity leading to decrease in steatosis in the hepatocyte. Garlic lectin might also have similar action on HMG-CoA leading to decrease in in steatosis.

Table 1: The mean ($\pm$ SE) histopathological score values of liver of different groups.

Liver lesions	Group I (NC)	Group II (DC)	Group III (RC)	Group IV (GL 10)	Group V (GL 20)	Group VI (GL 40)
Hepatocyte swelling	0 ^a	2.83 $\pm$ 0.3 ^c	0.66 $\pm$ 0.21 ^b	1.08 $\pm$ 0.27 ^b	0.75 $\pm$ 0.17 ^b	0.83 $\pm$ 0.16 ^b
Microvesicular steatosis	0.33 $\pm$ 0.12 ^a	4.08 $\pm$ 0.2 ^d	1.91 $\pm$ 0.2 ^b	2.66 $\pm$ 0.16 ^c	1.66 $\pm$ 0.2 ^b	1.58 $\pm$ 0.24 ^b
Macrovesicular steatosis	0 ^a	3.5 $\pm$ 0.22 ^d	1.16 $\pm$ 0.16 ^b	2.08 $\pm$ 0.08 ^c	1.41 $\pm$ 0.15 ^b	1.08 $\pm$ 0.2 ^b
Fatty cyst formation with amorphous eosinophilic material	0 ^a	4.16 $\pm$ 0.3 ^c	1.33 $\pm$ 0.16 ^b	1.91 $\pm$ 0.15 ^b	1.16 $\pm$ 0.25 ^b	1.01 $\pm$ 0.16 ^b
Kupffer cell hyperplasia	0 ^a	3.33 $\pm$ 0.42 ^d	1.45 $\pm$ 0.1 ^b	2.5 $\pm$ 0.42 ^c	1.5 $\pm$ 0.23 ^b	1.33 $\pm$ 0.21 ^b
Single cell necrosis	0.23 $\pm$ 0.14 ^a	4.1 $\pm$ 0.25 ^d	1.16 $\pm$ 0.1 ^b	2.2 $\pm$ 0.11 ^c	1.33 $\pm$ 0.21 ^b	1.23 $\pm$ 0.24 ^b
Centrilobular necrosis	0 ^a	3.33 $\pm$ 0.42 ^d	1.91 $\pm$ 0.3 ^b	2.08 $\pm$ 0.15 ^c	1.5 $\pm$ 0.24 ^b	1.41 $\pm$ 0.27 ^b
Bile duct proliferation	0 ^a	3.16 $\pm$ 0.3 ^c	1.66 $\pm$ 0.33 ^b	1.91 $\pm$ 0.27 ^b	1.83 $\pm$ 0.16 ^b	1.33 $\pm$ 0.21 ^b
Fibrosis	0 ^a	2.25 $\pm$ 0.17 ^c	0.83 $\pm$ 0.16 ^b	1.16 $\pm$ 0.30 ^b	1 $\pm$ 0.31 ^b	0.93 $\pm$ 0.27 ^b

**Fig 1:** Liver from a normal control rat showing normal architecture and size.**Fig 4:** Liver from a treatment Group IV rat showing focal necrotic spot**Fig 2:** Liver from a disease control (Group II) rat showing moderate enlargement with rounded border, no lustre and yellowish discoloration of parenchyma with thickened capsule.**Fig 5:** Liver from a treatment Group V rat showing minimal enlargement with lustre and normal rounded border and yellow discolouration.**Fig 3:** Liver from a reference control (Group III) rat showing mild enlargement with normal lustre and borders.**Fig 6:** Liver from a treatment Group VI rat showing minimal enlargement and normal rounded border.

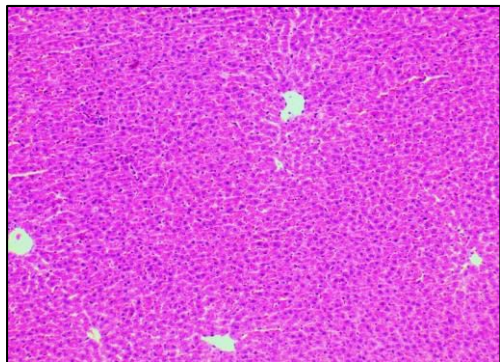


Fig 7: Section of liver from a normal control rat (Group I) showing hepatic lobule made up of radiating plates of strands of cells forming a network around the central vein with narrow sinusoids. (H&E X100)

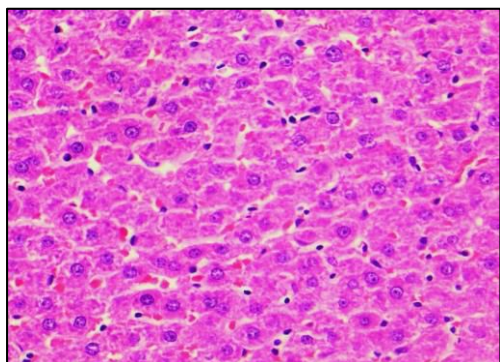


Fig 8: Section of liver from a normal control rat (Group I) showing normal hepatic cells with well preserved cytoplasm and nucleus. (H&E X400)

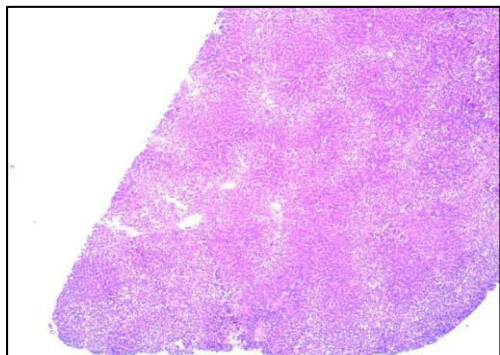


Fig 7: Section of liver from a normal control rat (Group I) showing hepatic lobule made up of radiating plates of strands of cells forming a network around the central vein with narrow sinusoids. (H&E X100)

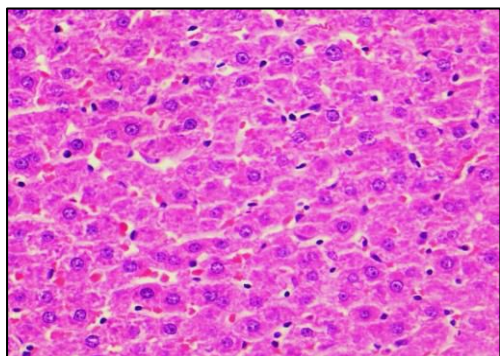


Fig 8: Section of liver from a normal control rat (Group I) showing normal hepatic cells with well preserved cytoplasm and nucleus. (H&E X400)

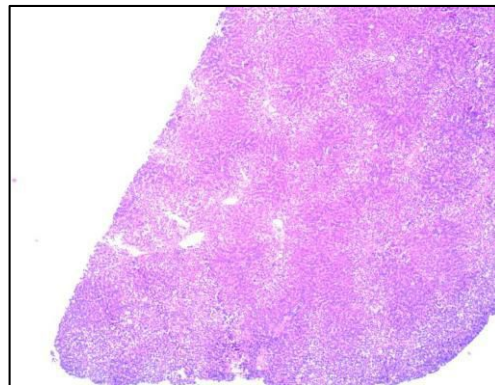


Fig 9: Section of liver from a disease control rats showing centrilobular necrosis leading to mottled appearance. (H&E X40)

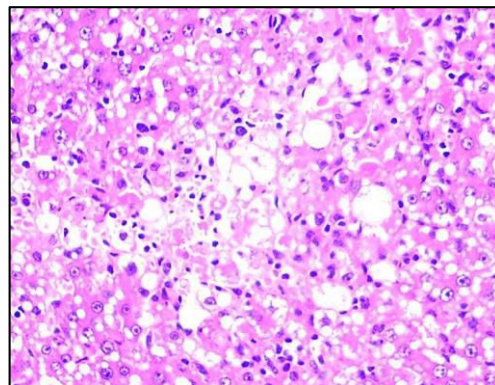


Fig 10: Section of liver from a disease control rat showing severe macrovesicular steatosis. (H&E X400)

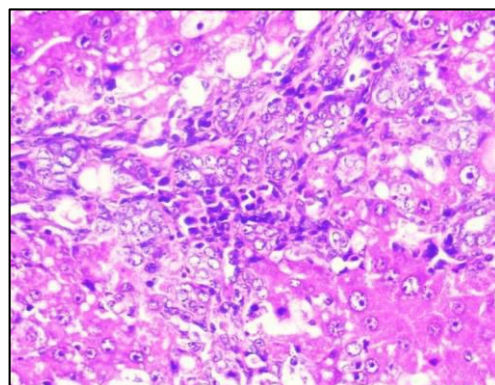


Fig 11: Section of liver from a disease control rat showing severe bile duct proliferation around portal triad. (H&E X400)

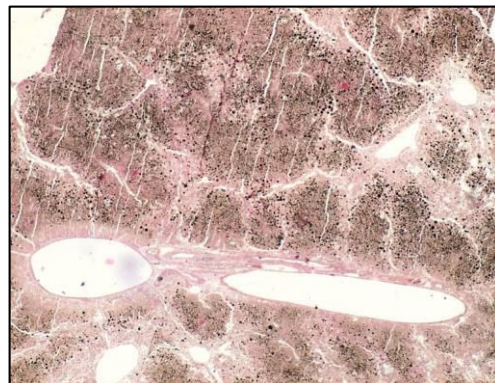


Fig 12: Section of liver from a disease control rat showing black coloured lipid infiltration in the hepatocytes around the centrilobular zone. (Osmium tetroxide X40).

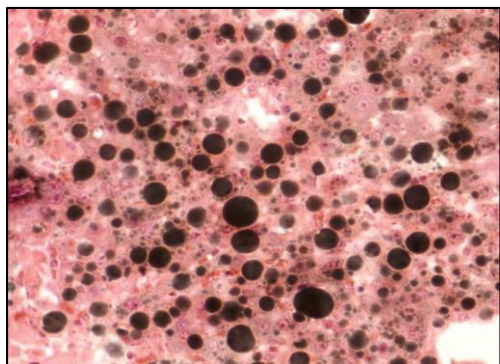


Fig 13: Section of liver from a disease control rats showing lipid infiltration in the hepatocytes appearing black when stained with osmium tetroxide. (Osmium tetroxide X400)

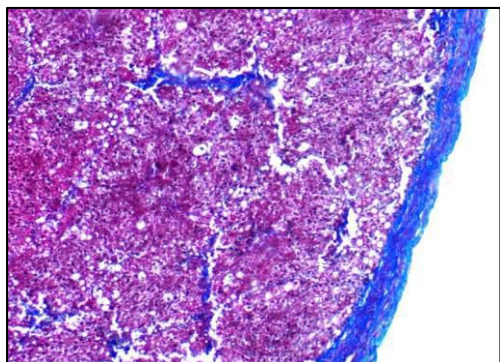


Fig 14: Section of liver from a disease control rat showing peri-hepatic fibrous capsule thickening and interstitial fibrosis characterised by blue coloured collagen fibres in parenchyma. (MST X100)

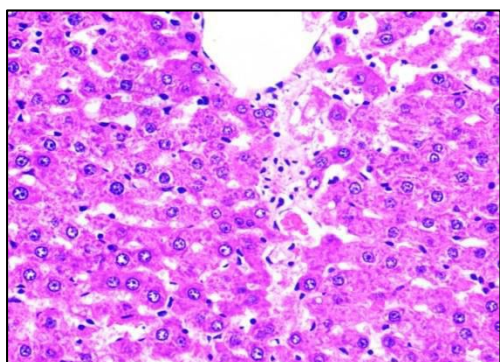


Fig 15: Section of liver from a reference control rat showing centrilobular degeneration and macrovesicular steatosis fused to form fatty cyst of lesser diameter compared to Group II with amorphous eosinophilic material inside. (H&E X400)

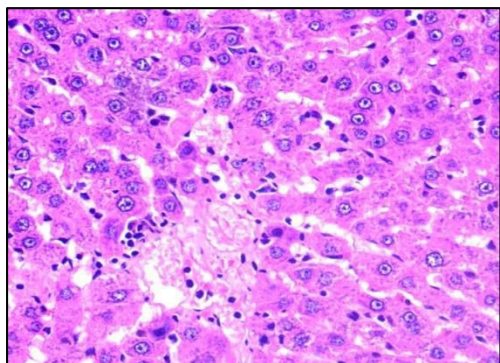


Fig 16: Section of liver from a Group IV rat showing degenerating hepatocytes and macrovesicular steatosis fused to form fatty cyst of lesser diameter compared to Group II. (H&E X400)

Acknowledgments

There is no conflict of interest related to the work.

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