HISTOLOGICAL CHANGES IN VITAL ORGANS OF RATS EXPOSED TO CCl₄ AND TREATED WITH AN EXTRACT OF PLEUROTUS OSTREATUS

1. Introduction

Histopathological studies are of relevance in providing direct evidence of the tissue toxicity of a chemical and in demonstrating the tissue-protective effect of a drug; the results of such studies generally support the results of biochemical evaluation. Histological studies have been performed to directly demonstrate the presence of injuries as a result of the hepatotoxicity of CCl₄ (Ashok-Shenoy et al., 2001; Lee and Jeong, 2002; Yadav and Dixit, 2003; Kaur et al., 2006). Many studies have demonstrated that extracts of various natural products rich in antioxidants can confer protection against CCl₄-induced injury in rat models. These include extracts of Ginkgo biloba (Ashok-Shenoy et al., 2001), Platycodi radix (Lee and Jeong, 2002), Kalanchoe pinnata (Yadav and Dixit, 2003), Cassia siamea (Kaur et al., 2006), Lygodium flexuosum (Wills and Asha, 2006), Phyllanthus maderaspatensis (Asha et al., 2007) and Passiflora alata (Rudnicki et al., 2007).

The mushroom Pleurotus ostreatus has been evaluated as a source of antioxidant in conferring protection against oxidative stress. This has been demonstrated by various biochemical investigations conducted in a state of acute oxidative stress that is CCl₄–induced toxicity (Jayakumar et al., 2006), as well as in a state of chronic stress, that is ageing (Jayakumar et al., 2007). In the present investigation, histoarchitectural studies were performed on the liver, kidneys, heart and brain tissues of normal rats, rats exposed to CCl₄ (state of acute oxidative stress) and rats exposed to CCl₄ and treated
with an extract of *P. ostreatus*. This phase of the study was undertaken to provide histological correlates of observed changes in biochemical parameters.

2. Material and Methods

2.1 Chemicals

Physiological saline (0.9%)

0.9 g of sodium chloride in 100 ml of distilled water.

Bouin’s fluid

Prepared by adding 75 ml of saturated picric acid, 25 ml of formaldehyde and 5 ml of glacial acetic acid and filtered.

Harris haematoxylin

It was prepared by dissolving 1 g of haematoxylin in 10 ml of absolute alcohol. 20 g of ammonium alum, previously dissolved in 200 ml of hot water was added to the above. This solution was quickly boiled and 0.5 g of mercuric oxide was added to it upon which the solution turned to dark purple colour. It was cooled rapidly under the tap and filtered before use. 8 ml of glacial acetic acid was added after cooling to sharpen nuclear staining.

Eosin

0.5 g of water soluble eosin powder was dissolved in 30 ml of distilled water and made up to 100 ml with 70 ml of alcohol.

2.2 Animal Experiments

The male albino Wistar rats (150 -200 g) were acclimated for 20 days prior to dosing, during which time they had free access to food and water *ad libitum*. Eighteen
such acclimated rats were randomly divided into 3 groups of six each: Group I (normals) received only vehicle (olive oil; 1ml/kg b.w) for 4 days; Group II (toxin controls) received vehicle on the first and fourth days, and vehicle and CCl₄ (50% solution of CCl₄ in olive oil, 2 ml/kg b.w) on the second and third days; Group III (test rats) received mushroom extract (200 mg / kg b.w.) on the first and fourth days and mushroom extract and CCl₄ on the second and third days. All administrations were made intraperitoneally. On the fifth day, rats were sacrificed by decapitation. Immediately after sacrifice, the liver, kidneys, heart and brain were dissected out and were blotted free of blood and mucus, washed thoroughly in physiological saline.

2.3. Procedure

Tissues were then cut into pieces of desired size and fixed in Bouin’s fluid fixative immediately after autopsy. Fixation was carried out at room temperature for 24 hr, after which the tissues were transferred to 70 % alcohol. Several changes of 70% alcohol were given until the yellow colour disappeared from the tissues. The tissues were then dehydrated by passing through ascending grades of alcohol (30%, 50%, 70%, 90% and 100%), cleared in methyl salicylate and infiltrated with wax at 57°C. The tissues thus cleared were embedded in the paraffin.

Conventional techniques of paraffin – wax sectioning and haematoxylin – eosin staining were used for histological studies (Drury and Wallington, 1980). Paraffin sections of 6 to 8 µm thickness were cut using a rotary microtome (Leica, Germany). The sections, thus obtained, were stained in Harris’ haematoxylin and eosin and then washed in 90% alcohol for few seconds. The stained sections were dehydrated in 100% alcohol, cleared in xylene and mounted in DPX mountant. The stained slides were
observed in a Carl Zeiss (Germany) Axio 2 Plus Research Microscope. Images were captured through a CCD camera in a computer and processed using Carl Zeiss Axiovision software.

3. Results

3.1. Liver

Histological sections of the liver of normal (Group I) rats revealed masses of epithelial cells or hepatocytes (H) with a definite cell membrane and prominent nucleus (N); these were polygonal in shape, with six or more surfaces, and had a clearly defined cell membrane. The hepatocytes were found to constitute about 80% of the cell population in the liver. The surfaces were found to be either external or related to a sinusoidal space (SS), or closely applied to the surface of an adjacent liver cell to form a bile canaliculus. The nuclei were found to be spherical or ovoid, with a regular surface; each nucleus was vesicular in type, with prominent, scattered chromatin granules. Erythrocytes (E) were present in sinusoidal space (SS). A central vein (C) was found in the hepatic lobule (Fig. 37a & b).

In H and E- stained sections of liver of rats exposed to CCl₄ (Group II) which received no treatment the following alterations were noted. The lattice nature of the hepatocytes were disrupted; the hepatocyte cell membrane was damaged; the sinusoidal blood spaces were obliterated; the vascular system of the liver was disrupted, as evidenced by the disintegrated central vein and damaged hepatic sinusoids (Fig. 37c & d). However, the sections of liver of CCl₄- exposed rats that had been treated with mushroom extract (Group III) revealed only minimal damage to blood capillaries and obliteration of sinuses; the rest of tissues appeared normal (Fig. 37 e & f).
**Fig. 37. Histoarchitecture of the liver in different groups of rats**

Fig. a  Histology of liver of normal rat. Hepatocytes and central vein seen clearly (Haematoxylin and Eosin (H&E) stain × 100).

Fig. b  Histology of liver of normal rat. Normal hepatocyte with prominent nucleus and erythrocytes in the sinusoidal space are clearly seen (H & E, × 400).

Fig. c  Histology of liver of CCl₄-intoxicated rat. Disintegrated central vein seen (H & E × 100).

Fig. d  Histology of liver of CCl₄-intoxicated rat. Disrupted hepatocytes, damaged hepatocyte cell membrane and obliterated sinusoidal spaces seen (H & E, × 400).

Fig. e  Histology of liver of CCl₄-intoxicated rat treated with mushroom extract. Partially damaged hepatocytes and central vein seen (H & E, × 100).

Fig. f  Histology of liver of CCl₄-intoxicated rat treated with mushroom extract. Normal hepatocytes and sinusoidal spaces seen (H & E, × 400).

C- central vein
H- hepatocytes
N- nucleus
SS- sinusoidal space
3.2. **Kidney**

Sections of the kidneys (H & E-stained) of the normal rats exposed prominent renal corpuscles (R). Each corpuscle was found to possess a vascular (V) and urinary (U) pole. At the vascular pole, the afferent arteriole (A) was seen to break up into glomerular capillaries filled with erythrocytes. Proximal (P) and distal (D) convoluted tubules appeared normal in the kidneys of Group I rats (Fig. 38 a & b). H and E sections of the kidneys of CCl4-exposed untreated rats revealed distorted renal corpuscle and distented proximal and distal convoluted tubules (Fig. 38c & d). In CC4-exposed rats treated with mushroom extract, most of the features were normal, except for partial disruption of the renal corpuscles and slight enlargement of the capsular space (Fig. 38 e & f).

3.3. **Heart**

H and E - stained tissue sections of the heart of normal rats revealed the following structural details: i) muscle fibers or trabeculae (T) that were mainly parallel in alignment but which also exhibited branching and cross-bridging in a pseudosyncitial arrangement. ii) lying between the trabeculae, numerous blood capillaries (C), lined by endothelium, was noted; some endothelial nuclei (N) were also seen, manifesting as large nuclei, located centrally in the fibers with nonfibrillar sarcoplasm at their pole. iii) Intercalated discs (ID) (the sites of cell junction) were seen, appearing as dark lines crossing fibres either transversely (TV) or in a staggered, zigzag manner (ZZ) (Fig. 39 a & b).

In H and E - stained tissue sections of the heart of CCl4-exposed untreated rats, disruption of blood capillaries was noted; this disruption led to liberation of blood cells,
Fig. 38. Histoarchitecture of the kidneys in different groups of rats

Fig. a Histology of kidneys of normal rat. Normal renal corpuscles clearly seen; proximal and distal convoluted tubules seen (H & E, × 100).

Fig. b Histology of kidneys of normal rat. Normal renal corpuscles prominently seen; these possess a vascular and urinary pole, afferent arteriole with capsular space, and proximal and distal convoluted tubules (H & E, × 400).

Fig. c Histology of kidneys of CCl₄-intoxicated rat. Distorted renal corpuscles and distended proximal and distal convoluted tubules seen (H & E, × 100).

Fig. d Histology of kidney of CCl₄-intoxicated rat. Distorted renal corpuscles and distended proximal and distal convoluted tubules seen (H & E, × 400).

Fig. e Histology of kidney of CCl₄-intoxicated rat treated with mushroom extract. Slightly distended proximal and distal convoluted tubules seen (H & E, × 100).

Fig. f Histology of kidney of CCl₄-intoxicated rat treated with mushroom extract. Enlargement of capsular space seen (H & E, × 400).

R- renal corpuscle
U- urinary pole
P- proximal convoluted tubules
D- distal convoluted tubules
N- nucleus
V- vascular pole
Fig. 39. Histoarchitecture of the heart in different groups of rats

Fig. a Histology of cardiac muscle of normal rat. Normal muscle trabeculae seen (H & E, × 100).

Fig. b Histology of cardiac muscle of normal rat. Normal muscle fibers or trabeculae with cross-bridges, endothelium with endothelial nuclei, transverse and zig zag intercalated discs seen (H & E, × 400).

Fig. c Histology of cardiac muscle of CCl₄-intoxicated rat. Disrupted blood capillaries, are seen, with liberated blood cells lying free in the spaces between trabeculae (H & E, × 100).

Fig. d Histology of cardiac muscle of CCl₄-intoxicated rat. Disrupted blood capillaries, are seen, with liberated blood cells lying free in the spaces between trabeculae (H & E, × 400).

Fig. e Histology of cardiac muscle of CCl₄-intoxicated rat treated with mushroom extract. Normal trabeculae and blood capillaries seen (H & E, × 100).

Fig. f Histology of cardiac muscle of CCl₄-intoxicated rat treated with mushroom extract. Normal trabeculae and blood capillaries seen (H & E, × 400).

T- trabeculae
C- blood capillaries
ZZ- zig zag intercalated discs
N- nucleus
which were seen to be lying free in the spaces between trabeculae (Fig. 39 c & d). However, CCl₄-exposed rats that had been treated with mushroom extract, the cardiac tissue exhibited only minor damage to the blood capillaries; the rest of the tissues appeared normal (Fig. 39 e & f).

3.4. Brain

H and E-stained tissue sections of the brain (cerebral cortex) of normal rats revealed the presence of nerve cell fibers, neuroglia and blood vessels; the cells were found to vary in morphology from pyramidal to stellate to fusiform and were arranged in a laminated manner (Fig. 40 a& b). In H and E-stained sections of brain of CCl₄-exposed untreated rats, persistence of vacuolization of the cerebral cortical cells was a notable finding (Fig. 40 c). In CCl₄-exposed rats that had been challenged with mushroom extract, the vacuolization seen in the cerebral cortical cells was significantly reduced (Fig. 40 d).

4. Discussion

Histopathological studies were performed to provide direct evidence of the tissue toxicity of CCl₄, and of the tissue protective effect of the extract of Pleurotus ostreatus. Marked disruption of the structure of hepatocytes was noted in liver tissue of Group II rats (exposed to CCl₄ alone). Only minimal disruption of the structure of hepatocytes was noted in liver tissue of Group III rats (exposed to CCl₄ and mushroom extract); this minimal disruption of the hepatocyte structure complemented the results of the liver enzyme studies in chapter II (SGOT, SGPT and SALP activities and MDA levels approximated to the levels in normal rats).
Fig. 40. Histoarchitecture of the brain in different groups of rats

Fig. a Histology of cerebral cortex of normal rat. Outer gray matter with pyramidal neurons and inner white matter seen (H & E, × 100).

Fig. b Histology of cerebral cortex of normal rat. Normal nerve cell fibres and neuroglia seen (H & E, × 400).

Fig. c Histology of cerebral cortex of CCl₄-intoxicated rat. Vacuolization seen in cortical cells (H & E, × 400).

Fig. d Histology of cerebral cortex of CCl₄-intoxicated rat treated with mushroom extract. Normal cerebral cortex seen (H & E, × 400).

G- gray matter
W- white matter
PN- pyramidal neurons
CC- cortical cells
N- neuroglia
Administration of CCl₄ to rats has been shown to result in various histoarchitectural changes; these include centrilobular hepatocytes with single cell necrosis surrounded by neutrophils and congestion of the central vein and sinusoids with acute and chronic inflammatory cells (Ashok Shenoy et al. (2001). In addition, ballooning degeneration culminating in severe necrosis (Venukumar and Latha, 2004; Kaur et al., 2006; Rudnicki et al., 2007), damaged hepatocytes (Asha et al., 2007), and inflammatory cell infiltration, fatty degeneration and hydropic degeneration (Wu et al., 2007) have been noted in the liver of rats exposed to CCl₄.

In the present investigation, in addition to liver damage, distortion of the renal corpuscles and distention of the proximal and distal convoluted tubules in the kidneys, disruption of blood capillaries in the heart and vacuolization of the cerebral cortical cells in the brain tissue were observed in CCl₄-exposed untreated rats. These observations suggest that the liver is not the only target organ of CCl₄; CCl₄ can apparently affect other tissues such as the kidneys, heart and brain as well. The impact of CCl₄ on several organs has been reported earlier (Ahmad et al., 1987; Ohta et al., 1997; Ozturk et al., 2003).

Rats treated with an extract of Lygodium flexuosum, a potential antioxidant, have been reported to recover from CCl₄-induced centrilobular necrosis and bridging hepatic necrosis (Wills and Asha, 2006). Treatment with an extract of the leaf of Passiflora alata, a source of antioxidant, appears to prevent CCl₄-induced histological changes in the liver of rats in a dose-dependent manner, as suggested by mild hepatocellular necrosis and moderate inflammatory cell infiltration (Rudnicki et al., 2007). The hepatic tissue of rats treated with an extract of Phyllanthus maderaspatensis
as a source of antioxidant was found to recover from CCl₄-induced damage, as
evidenced by the presence of normal hepatocytes with well-defined nuclei (Asha et al.,
2007). Pretreatment with an extract of the flower of *Cassia siamea*, a potent
antioxidant, has also been reported to preserve the hepatic architecture, with only a few
areas of hemorrhage between columns of hepatocytes in rats exposed to CCl₄ (Kaur et
al., 2006). In addition, administration of the antioxidant compound echinacoside has
been reported to ameliorate CCl₄-induced liver injuries (Wu et al., 2007). So also, in
the present study, administration of an extract of the oyster mushroom *Pleurotus
ostreatus*, as a source of antioxidant, appeared to prevent CCl₄-induced tissue damage
in all vital organs; histopathological studies revealed that the hepatocytes and
sinusoidal spaces of the liver, renal corpuscles of the kidneys, trabeculae of the heart
and cortical cells of the brain were all normal in architecture.

5. Conclusion

Administration of an extract of the oyster mushroom *Pleurotus ostreatus* as a
source of antioxidant appeared to protect the liver, kidneys, heart and brain of Wistar
rats from CCl₄-induced acute oxidative stress by preventing structural alterations such
as disruption of the structure of hepatocytes and sinusoidal spaces of the liver, renal
corpuscles of the kidneys, trabeculae of the heart and cortical cells of the brain. These
observations also suggest that the liver is not the only target organ of CCl₄, for it
appears to cause damage in other organs as well.