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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(5): 46-56

www.thepharmajournal.com
Received: 04-03-2020
Accepted: 06-04-2020

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Cisplatin induced histopathological changes in the liver and its amelioration by *Andrographis paniculata*

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DOI: https://doi.org/ 10.22271/tpi.2020.v9.i5b.4627

Abstract
The role of ethanolic extract of *Andrographis paniculata* [AP], in preventing cisplatin [CP] induced histopathological changes in rat hepatic tissue was studied. Sixty Wistar Albino rats were divided into five groups with twelve rats in each group. Group I served as normal control group. To group II rats, CP was administered at 7.5 mg/kg body weight intraperitoneally for single dose. Rats in group III served as AP control and were administered AP at the dose of 500 mg/kg body weight for 45 days. Group IV rats were pre-treated with AP fifteen days prior to CP administration and followed by AP treatment for 45 days. Rats in group V were administered with CP and concurrently treated with AP extract at 500 mg/kg by oral gavaging for 45 days. The liver samples collected at 7th, 14th, 28th, and 45th day of the study were subjected for histopathological studies. Results documented that, CP produced severe congestion and dilation of central vein, portal vessels, sinusoids congestion and dilation, presence of necrotic and apoptotic cells and polymorphonuclear cell infiltration in the liver. The AP treatment groups revealed improvement in the liver architecture, while pretreatment of AP produced the much earlier amelioration of the pathological changes. The study indicated that, prior administration of AP inhibited the CP injury and has good prophylactic effect.

Keywords: Andrographis paniculata, cisplatin, histopathology, necrosis, apoptotic cells

1. Introduction
Cisplatin (CP) is one of the antineoplastic drugs used for the different kinds of cancer. It is a platinum containing anticancer drug and it is a member of heavy metal alkylating agent [1]. It stops the growth of the cells by the formation of DNA adducts and cross links, there by cell cycle arrest and finally triggering apoptosis [2]. It does not distinguish between malignant and normal fast-growing cells, so it eliminates both types of cells [3]. This drug has several toxic effects that interfere with therapeutic efficacy. The nephrotoxicity of cisplatin is recognized as the most important dose limiting factor, but high doses of cisplatin also induces hepatotoxicity [4]. The exact mechanism of the CP induced hepatotoxicity is not well known however, cisplatin preferentially taken up and accumulated in the liver and kidney cells [5]. Along with interaction with DNA, cisplatin also stimulates oxidative stress is one of the important mechanisms involved in cisplatin-induced hepatotoxicity resulting in the enhanced production of reactive oxygen species, reduction in the mitochondrial membrane potential and decrease in antioxidant enzymes [6].

Over the past few years, the use of indigenous drugs has been on the rise along with modern system of medicine due to lesser side effects. *Andrographis paniculata* (AP) is one such important medicinal plant widely used around the world. It is commonly known as “Kalmegh” in India and it is widely cultivated in southern Asia. Most commonly, the leaves and roots have been traditionally used over the centuries for different medicinal purposes [7]. AP has been reported to possess a wide range of pharmacological activities such as antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, hepato-protective, nephroprotective, immunomodulatory due to presence of variety of phytochemicals [8]. Phytochemical analysis has revealed that, AP is rich source of alkaloids, phenolics, tannins, diterpenes such as andrographalide, neoandrographalide, deoxy-andrographalide, homoandrographalide and andrpgraphan [9]. Thus, the aim of the present work to study the effect of CP on liver tissue of rat histologically and on other hand to investigate the role of AP in experimentally induced hepatotoxicity.

2. Materials and Methods

2.1 Dugs and chemicals

Cisplatin [Kemoplat] was procured from Fresenius Kabi India Pvt. Ltd. Pune, India. and the ethanolic extract of *Andrographis paniculata* (AP) was obtained from Himalaya Herbal Pvt Ltd. Bangalore, India

2.2 Animals

Normal adult Wistar albino rats weighing approximately 180-200 grams were procured from commercial animal facility, Bangalore for the study. They were maintained under standard laboratory conditions and fed with *ad libitum* standard commercial rat feed and clean drinking water. The duration of experiment was for a period of 45 days and a prior permission was obtained from the Institutional Animal Ethics Committee [IAEC] for the conduct of the experiment.

2.3 Experimental design

The rats were maintained under standard laboratory conditions for a period of 15 days for acclimatization in the experimental animal house. The rats were divided, based on the body weight, into five groups with twelve rats in each group.

Group I: Negative control - injected with 0.5ml sterile PBS intraperitoneally on Day 1 and gavaged with PBS daily.

Group II: Positive control- hepatotoxicity induced with administration of cisplatin at 7.5 mg/kg body weight intraperitoneally for single dose.

Group III: *Andrographis paniculata* control-animals supplemented with ethanolic extract of *Andrographis paniculata* alone at the dose rate of 500 mg/kg body weight.

Group IV: Supplemented with *Andrographis paniculata* extract at the dose rate of 200mg/kg bodyweight 15 days prior to induction of hepatotoxicity by CP.

Group V: Supplemented with *Andrographis paniculata* extract at the dose rate of 500 mg/kg bodyweight concurrently with administration of CP.

2.4 Histopathology

To study the progressive effects of the treatments given to different groups, rats from each group were sacrificed humanely under ketamine hydrochloride on 7th, 14th, 28th, and 45th day of post induction of hepatotoxicity. Further, the representative liver tissues samples of 3-5 mm thickness were collected and preserved in 10 per cent neutral buffered formalin for 48hrs and the tissues were processed by the routine paraffin embedding technique and sections of 4µm thickness were cut using a microtome and subjected to routine hematoxylin and eosin (H&E) staining [10].

3. Results and Discussion

The liver in control group (Group I) and AP control group (Group III) showed apparently normal microscopical architecture throughout the study period (Fig. 1).

In cisplatin control group (Group II) on 7th day of the experiment, liver was severely congested with severe dilation of portal vessels and central veins (Fig. 2). Sinusoidal spaces were also dilated and congested. The hepatic parenchyma revealed presence of both single cell necrosis as well as apoptotic cells (Fig. 3). The necrotic cells which occurred singly or in small groups showed loss of membrane, hazy cytoplasm and either pyknotic or karyorrhectic nucleus. The apoptotic cells revealed either condensed or fragmented nucleus, eosinophilic cytoplasm with intact cell membrane. The subendothelial hepatocytes of several central veins were necrotic and the adjacent rims of hepatocytes were atrophic with condensed nucleus and darkly stained cytoplasm. Perivascular haemorrhages were also observed in several central veins. Mild proliferation of bile duct epithelial cells (Fig. 4) was observed in the portal triads along with mild infiltration of inflammatory cells. On 14th day also liver showed severe congestion of portal vessels and central veins with severe dilation and congestion of sinusoidal spaces (Fig. 5) and most of the portal triads showed endothelial injury with thrombotic angiopathy, especially of portal vessels (Fig. 6). Perivascular haemorrhage and oedema were also observed. The hepatocytes in the areas of severe congestion were degenerated and necrotic with vacuolated cytoplasm indicating ischaemic injury. Multifocal areas of necrosis were also observed with infiltration of polymorphic inflammatory cells (Fig. 7). Subcapsular hepatocytes were also necrotic with infiltration of inflammatory cells. In addition, scattered single cell necrotic and apoptotic cells were also observed (Fig. 5). On 28th day of the experiment, there was persistence of severe degree of congestion, perivascular haemorrhages and edema. A large number of hepatocytes revealed vacuolar degeneration and necrosis. Pre apoptotic cells which appeared either angular or shrunken with highly eosinophilic cytoplasm and partially condensed nucleus were observed around portal triad and central veins and also scattered in the parenchyma (Fig. 8). Apoptotic cells were also observed and distributed in the liver lobules. Mild connective tissue proliferation was observed in the portal triad with occasional mononuclear cell infiltration (Fig. 9). On 45th day, also there was persistence of congestion of venules and sinusoidal spaces which was reduced in severity. Multifocal areas of necrosis, single cell necrosis, apoptosis and mild connective tissue proliferation in the portal triad were also persistent however, in a reduced intensity compared to 28th day changes.

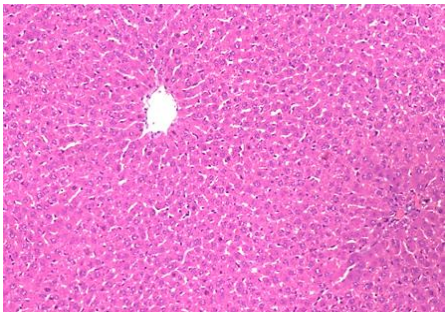

Fig 1: Liver from Group I showing normal architecture at 7th day of experiment. H&E ×100

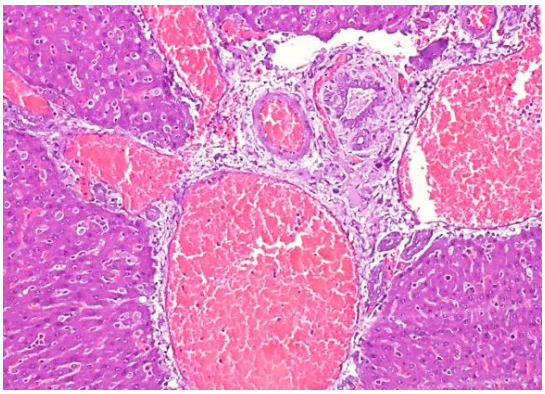

Fig 2: Section of liver from Group II rat at 7th day of experiment showing severe congestion of portal vessels. H&E ×100

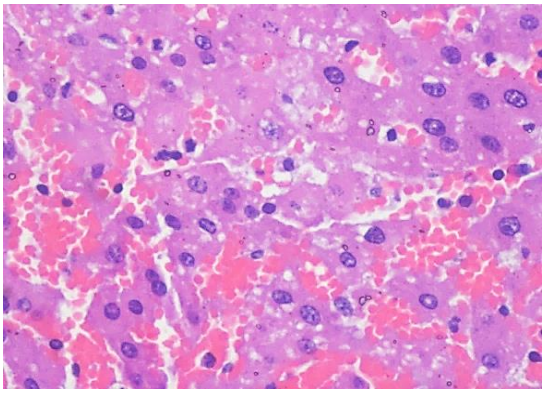

Fig 3: Section of liver from Group II rat at 7th day of experiment showing dilatation and congestion of the sinusoidal spaces and necrosis of hepatocytes. H&E ×400

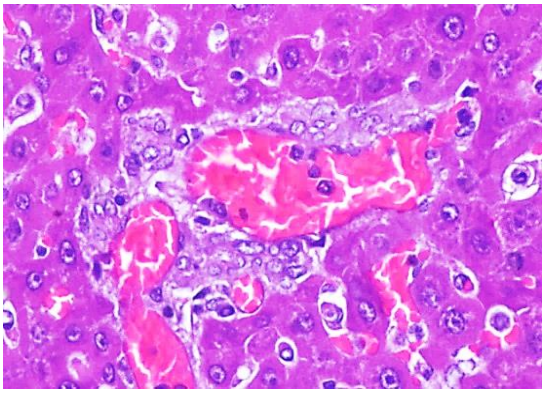

Fig 4: Section of liver from a Group II rat at 7th day showing mild bile duct epithelial proliferation and congestion. H&E ×400

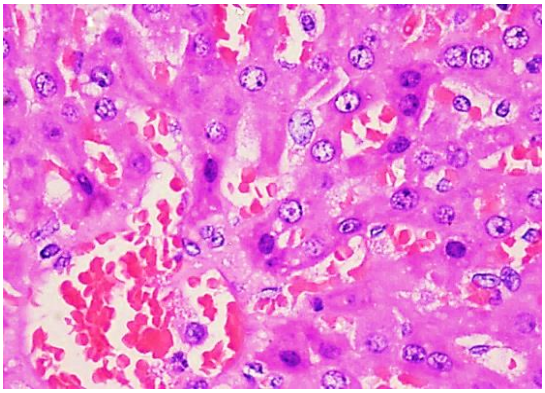

Fig 5: Section of liver from a Group II rat at 14th day of experiment showing congestion of central vein and sinusoidal spaces and presence of pre-apoptotic and necrotic hepatocytes. H&E ×400

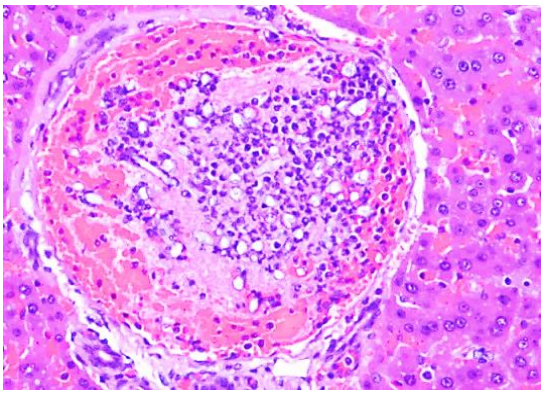

Fig 6: Section of liver from a Group II rat at 14th day of experiment showing endothelial injury with thrombotic angiopathy of portal vessel. H&E ×200

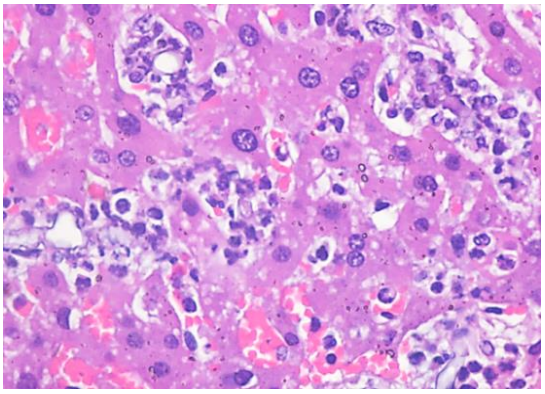

Fig 7: Section of liver from Group II rat at 14th day showing multifocal areas of necrosis with infiltrations. Note dilated and congested sinusoidal spaces. H&E ×400

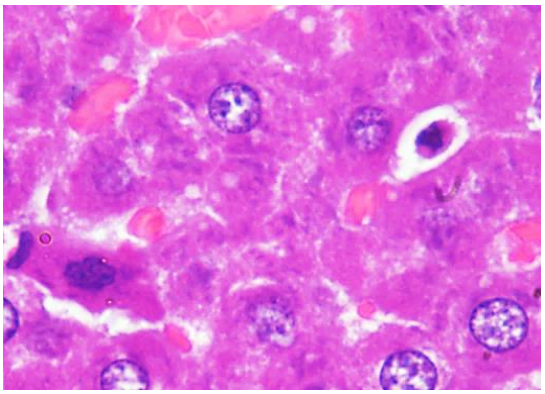

Fig 8: Section of liver from a Group II rat at 28th day showing presence of pre-apoptotic and apoptotic cells. H&E×1000

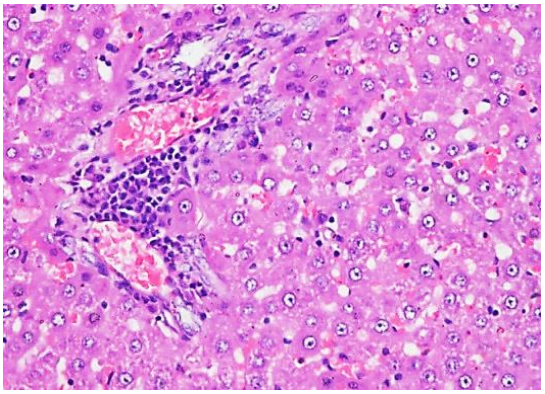

Fig 9: Section of liver from a Group II rat at 28th day of experiment showing mononuclear cell infiltration in the portal triad. H&E ×200

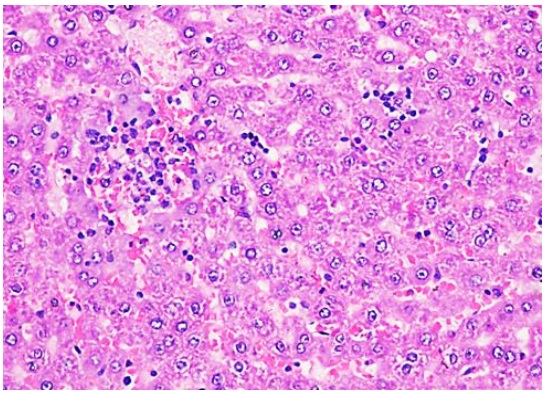

Fig 10: Section of liver from a Group IV rat at 7th day showing mild sinusoidal congestion, focal areas of necrosis with infiltration and mild vacuolar degeneration in hepatocytes. H&E ×200

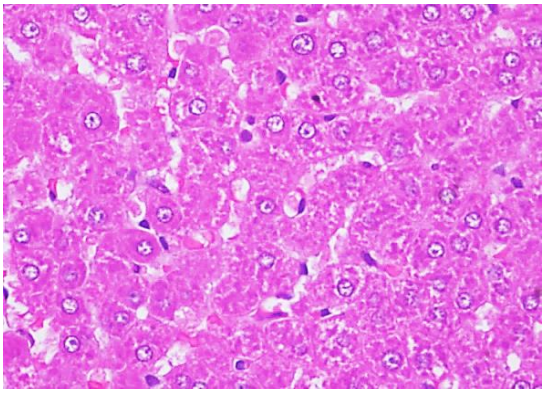

Fig 11: Section of liver from a Group IV rat at 7th day showing mild granular to vacuolar degeneration. H&E ×400

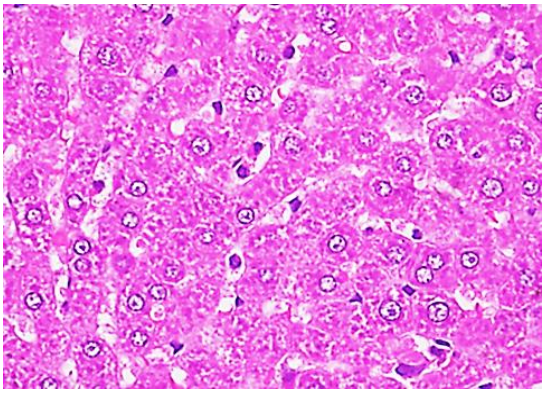

Fig 12: Section of liver from Group IV rat at 28th day showing mild granular to vacuolar degeneration and presence of occasional apoptotic cells. H&E ×400

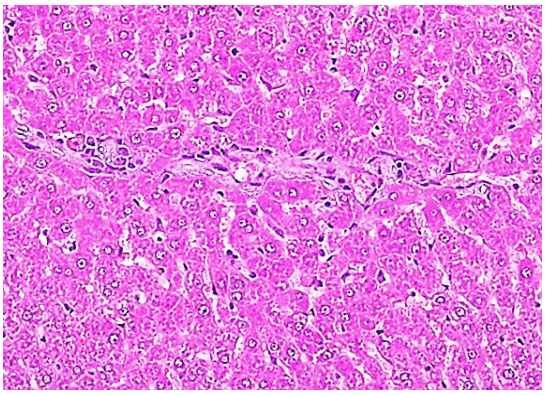

Fig 13: Section of liver from a Group IV rat at 45th day showing mild infiltration of mononuclear cells. H&E ×200

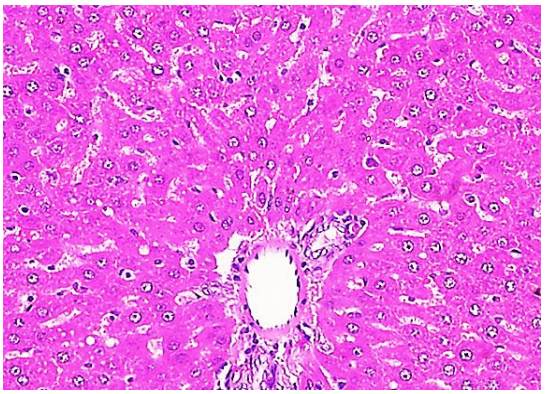

Fig 14: Section of liver from a Group IV rat at 45th day showing restoration of normal structure of liver. H&E ×200

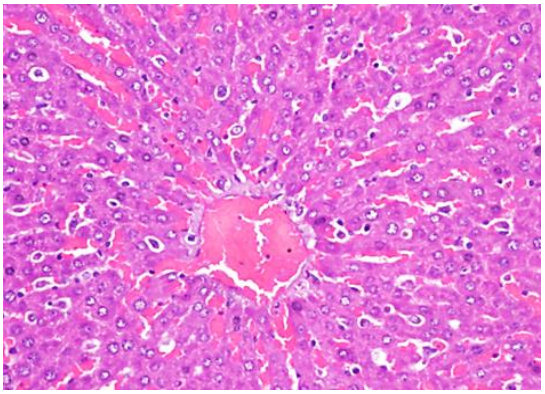

Fig 15: Section of liver from a Group V rat at 7th day showing congestion of central vein and sinusoidal spaces. H&E ×100

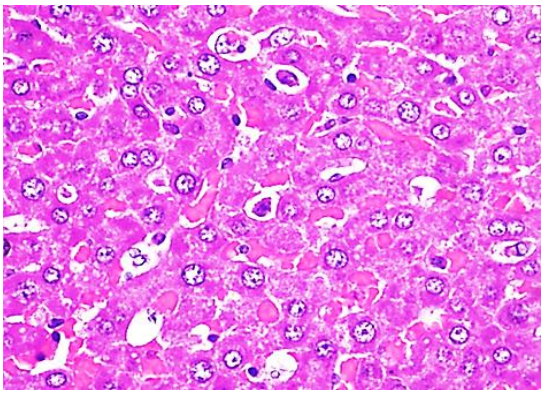

Fig 16: Section of liver from a Group V rat at 7th day of experiment showing congestion and single necrotic and apoptotic cells. H&E ×400

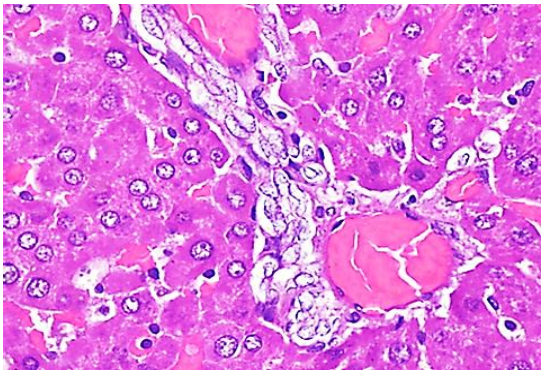

Fig 17: Section of liver from Group V rat at 28th day of experiment showing biliary epithelial proliferation with improved architecture of hepatocytes. H&E ×400

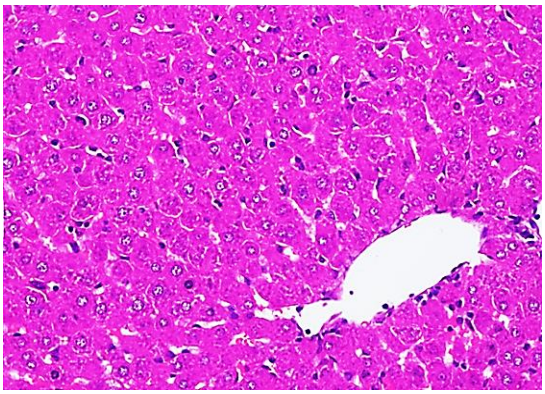

Fig 18: Section of liver from a Group V rat at 45th day of experiment showing improved architecture of hepatocytes. H&E ×400

Similar findings of microscopic changes in the liver due to cisplatin induced hepatotoxicity have also been reported in various earlier studies [8, 11-18]. The precise mechanism for cisplatin induced hepatotoxicity is not well understood. However, many studies documented that, cisplatin is preferentially taken up and accumulated in the liver cells second only to kidney cells causing cellular damage and inhibition of their division [5]. However, other mechanisms are also contributory such as oxidative stress pathways, inflammatory pathways, lipid peroxidation, suppression of enzymatic and non-enzymatic anti-oxidant levels such as glutathione, CAT, SOD and the elevation of malondialdehyde (MDA) in hepatic tissues [8, 12, 15, 19-23]. Furthermore, functional and structural mitochondrial injury, apoptosis, perturbation Ca^{2+} homeostasis, involvement of proinflammatory genes such as COX-2, and inducible nitric oxide synthetase (iNOS) may also play important role in the mechanism of cisplatin induced hepatotoxicity [2].

Another characteristic microscopical observation in the present study was presence of apoptotic cells in the liver. Apoptotic cells and apoptotic bodies in cisplatin induced hepatotoxicity and have been described in various earlier studies [8, 12, 24]. Cisplatin, as indicated earlier, cross links the DNA in several different pathways and forms DNA adducts, causing G2 cell cycle arrest and interferes with cell division by mitosis. The damaged DNA elicits DNA repair mechanisms, which in turn activate apoptosis when repair proves impossible [4, 25]. In addition, apoptosis in cisplatin hepatotoxicity could be due to increased oxidative stress with increased free radical injury, reduction in the mitochondrion membrane potential and proinflammatory cytokines or depletion of intracellular antioxidants [26, 27]. The increased oxidative stress may also cause structural changes in nucleus by causing DNA fragmentation and denaturation, which play a critical role in the initiation of apoptosis [24].

In AP pre-treatment group (Group IV) the microscopical observation of liver on 7th day revealed mild changes which included, mild congestion of vessels, mild granular to vacuolar degeneration, mild biliary epithelial cell hyperplasia, sparse infiltration of inflammatory cells in the portal triads, occasional focal necrosis, mild Kupffer cell proliferation and presence of apoptotic cells (Fig. 10, 11). On 14th day, the lesions observed in the liver were similar to those of 7th day. On 28th day, the microscopical changes included, mild to moderate congestion, multifocal areas of necrosis with infiltration of inflammatory cells, granular to vacuolar degeneration of hepatocytes (Fig. 12), increase in Kupffer cell activity, infiltration of mononuclears in moderate numbers in the portal triad, mild bile duct epithelial proliferation and presence of pre-apoptotic and apoptotic cells. On 45th day, there was restoration of the architecture of the liver, almost to the normalcy. However, there was presence of occasional pre-apoptotic and apoptotic cells, mild increase in the Kupffer cell number and mononuclear cells in the portal triad (Fig. 13, 14).

On 7th day, microscopically *Andrographis paniculata* concurrent treatment group (Group V) revealed all the changes observed in the cisplatin control group, however, in a reduced intensity. The lesions included congestion of vessels (Fig. 15), presence of scattered pre-apoptotic, single cell necrotic and apoptotic cells (Fig. 16), mild vacuolar degeneration of hepatocytes, mild biliary epithelial proliferation and increased Kupffer cell activity. On 14th day the lesions observed in liver were similar to those observed at 7th day of the experiment. On 28th day, the degree of severity of congestion was reduced. Mild degree of vacuolar degeneration of hepatocytes, necrotic cells, pre-apoptotic and apoptotic cells were still present however in a reduced number. In addition, mild biliary hyperplasia was also observed (Fig. 17). On 45th day, mild degree of congestion, single cell necrotic and apoptotic cells, mild biliary hyperplasia and occasional focal necrotic areas with infiltration of inflammatory cells were observed. The architecture of hepatic lobules appeared to be improved compared to 28th day (Fig. 18).

Microscopically, both *Andrographis paniculata* treatment groups (Group IV and V) revealed improvement in the architecture of liver by 45th day of experiment. During initial period of the experiment, though cisplatin induced lesions were observed in the *Andrographis paniculata* treated groups, they were significantly lesser in severity compared to those of cisplatin control group (Group II). When compared between Group-IV and V groups, the restoration of architecture was much earlier in pre-treatment group (Group IV) which indicated that pre-treatment of *Andrographis paniculata* inhibited the severity of cisplatin injury.

The hepatoprotective effect of *Andrographis paniculata* has been well established in several earlier studies in various chemical induced hepatic injury [28-34]. The hepatoprotective effect of the *Andrographis paniculata* could be attributed to the antioxidative, anti-inflammatory and free radical scavenging properties of various phytoconstituents of *Andrographis paniculata* especially the andrographolide, andrographiside and neoandrographolide [28].

Hepatoprotective effect of *Andrographis paniculata* against cisplatin toxicity is dose dependent and high doses of *Andrographis paniculata* has been reported to give better protection in mice [8].

Morphological investigation of the cultured hepatic cells treated with alcoholic extract of the AP showed results similar to that produced by Liv 52 and increased viability in cultured hepatocytes exposed to CCL_4 [35]. Andrographolide of *Andrographis paniculata* significantly induces the expression of CYP1A1 and CYP1A2 mRNAs in a concentration dependent manner [36]. Induction of drug metabolizing enzymes is considered as an adaptive response to a cytotoxic environment. *Andrographis paniculata* ethanol extract and andrographolide induce the expression of the glutathione transferase, a phase II biotransformation enzyme involved in detoxification of various classes of environmental carcinogens, in rat primary hepatocytes [37].

4. Conclusion

It can be concluded that, cisplatin when administered at the dose of 7.5mg/kg induced the hepatotoxicity as evidenced by the histopathology. On the other hand, AP treatment has beneficial and responsible for hepatoprotective properties. It is therefore, recommended this to be useful co-treatment for cisplatin during treatment of cancer.

5. Acknowledgment

Authors are thankful to the Head of the Department, Department of Veterinary pathology and Dean, Veterinary College, Bangalore, for providing facilities to carry out the present study.

6. References

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