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Hepatoprotective Action of *Andrographis peniculata* Against Cisplatin Induced Toxicity in Mice: A Histological Study.

Sant Suman Rastogi[1], Mandavi Singh[1]*, Mohan Kumar[2], Anand Mishra[1] and Uttam Shrestha[1].

[1]Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India.
[2]Department of Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India.

ABSTRACT

Platinum complex cisplatin (CP) is one of the most potent drugs, widely used in chemotherapy against neoplasia. It is known to exhibit hepatotoxicity. The medicinal plant *Andrographis peniculata* (AP), used in this study is well known to be hepatoprotective. So we take this experiment to see the effect of AP extract against cisplatin induced hepatotoxicity in mice. Swiss albino female mice, randomly divided into six different groups. Group I was controls. Group IInd received single dose of CP *intraperitoneally* in dose of 6 mg/ kg body weight. Group IIIrd mice were given low dose of AP (20 mg/ kg body weight) only whereas Group IV high dose of AP (50 mg/ kg body weight) for period of 8 days. Group V[th] and VIth received CP along with low and high dose of AP respectively. Mice were sacrificed on 9th day of treatments. Liver was dissected out, and processed for histopathological studies. Liver of CP treated mice exhibited hepatic and nuclear degeneration along with dilation of sinusoids and central vein. Administration of AP reduces toxic effect of cisplatin on liver. On the basis of the findings it can be concluded that *Andrographis peniculata* is beneficial against cisplatin induced hepatotoxicity in mice.

Key words: Platinum, Kalmegh, Liver, Malignant, Reactive oxygen species

Corresponding author

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INTRODUCTION

Cisplatin (c*is-diammine dichloroplatinum* II, CP) is one of the chemotherapeutic agent [1] which is used against different kinds of epithelial cancers. It is a chemical compound and member of the heavy metal alkylating-like agents [2]. When CP comes in contact with cells it stops growth by formation of covalent cross-linked adduct between DNA bases of the cell and platinum compound. In this way it binds with DNA and interferes with replication and transcription process. It does not distinguished between a malignant and normal fast growing cell so it eliminates both type of cells by exerting several toxicities as its side effects [3-6].

Hepatotoxicity is one of the major side effects of cisplatin produced at high doses exposure [7, 8]. Adverse effect of CP on membrane integrity of hepatocytes and altered activities of enzymes responsible for different metabolic pathways in hepatocytes is well documented (9). Histopathological changes in liver due to cisplatin exposure such as necrosis and degeneration of hepatocytes have also been reported [10,11]. The exact mechanism of CP induced hepatotoxicity is not known, however oxidative stress induced by CP might be one of the cause. CP induces oxidative stress in liver by enhancing the production of reactive oxygen species (ROS, like superoxide, hydrogen peroxide, hydroxyl ions and oxygen free radicals [12, 13] and nitrogen reactive species (NRS, like nitrate and nitrites). The imbalance between formation of ROS and RNS in metabolism leads to pathological consequences in the liver [14].

The plant of family Acanthaceae, *Andrographis peniculata* (Kalmegh, AP), native to India and China, has been used for many years in herbal medicine against hepatic disorders, such as liver cirrhosis, hepatitis, jaundice [15], liver congestion and in alcoholism [16]. AP contains therapeutically important active principle diterpene lactones including andrographolide, neoandrographolide, deoxyandrographolide and didehydroandrographolide in its aerial parts [17-19]. Extensive research in the past decade indicated that because of the presence of active principles, AP possesses a wide range of pharmacological activities, including antioxidative (20-22), anti-inflammatory [23-25] and hepatoprotective [24-30] property. In the previous studies it also has been observed that AP showed inhibitory effect on chemically induced cytotoxicity, lipid peroxidation, and oxidative stress of CCl_4 [31, 32], benzene hexachloride [33], paracetamol and galactosamine [26] induced liver damage but the action of AP against hepatotoxicity induced by any anticancer drug is not yet reported. So in the light of hepatoprotective mechanism of AP, in the present study it was hypothesized to evaluated the possible role of *Andrographis peniculata* against cispaltin induced hepatotoxicity in Swiss albino mice.

MATERIALS AND METHODS

Drug Preparation

Andrographis peniculata (AP: aerial parts- leaves and stems) was collected from the Ayurvedic garden of Institute of Medical Sciences, Banaras Hindu University, Varanasi. Collected aerial parts were air dried, powdered with the help of grinder and then processed for extraction in a soxhlet apparatus in the presence of solvent methanol (250 gm powder / 2.5 liter of methanol). After completion of extraction process, the extract was kept in incubator (for 3-6

days) to evaporate the methanol in order to obtain solidify extract. This extract was further used for oral administration after making the desired dilutions in distilled water. Cisplatin (CP) was purchased from Cipla under the trade name of "Cytoplatin-10" and dissolved in normal saline (0.9 %) for *intraperitoneal* administration.

Animals and Experimental Design

Mature Swiss albino female mice of weighing 24- 28 grams were obtained from the animal house of the department of Anatomy, Institute of Medical Sciences, Banaras Hindu University. They were acclimatized by following the period of 12 hours light/ dark cycle in animal house and fed with a standard rodent diet and water *ad libitum*. All experiments were performed under consideration of laws and guidelines and with approval of animal ethics committee of Institute of Medical Sciences, Banaras Hindu University, Varanasi.

Twenty four acclimatized female mice were randomly divided into four groups with six animals in each group. **Ist** group was control, received distilled water (D/W) for the period of 8 days and **IInd** group was treated with CP at the single dose of 6 mg/ kg body weight *intraperitoneally* (i.p.). Group **IIIrd** and **IVth** were treated with AP orally by oral gavage for the period of 8 days at the dose of 20 mg/ kg body weight and 50 mg/ kg body weight respectively. Groups **Vth** and **VIth** received CP (6 mg/kg body weight) for one day and AP 20 mg/kg and 50 mg/ kg body weight respectively for a period of 8 days. AP was started on the same day 2 hrs after CP administration.

Histopathology

All experimental mice were sacrificed on 9th day of treatment by inhalation of mild diethyl ether followed by cervical dislocation. Liver was dissected out from all mice, rinsed in normal saline and fixed in Bouine's solution followed by process for wax imbedding to cut 5 µ thick sections. The histopathological changes in the liver sections were assessed after it was stained by haematoxylin and eosin (H&E) [34]. Stained slides were analyzed by light microscopy (Nikon eclipse E200, Japan) and images were captured using digital camera (Nikon DS-Fi2) attached to the microscope.

RESULTS

Histopathological Evaluations

The liver of mice treated with drug vehicle was considered as control, showed normal liver architecture comprising of the central vein surrounded by hexagonal hepatocytes. These hepatocytes forms hepatic cords radiating from the central veins with proper spaces in-between hepatic cords (i.e. hepatic sinusoids), and fine arranged Kupffer cells. In addition to prominent mononuclear hepatocytes some dividing bi-nucleated cells with granular chromatin materials were also observed (Fig. 1A, 2A & 3A).

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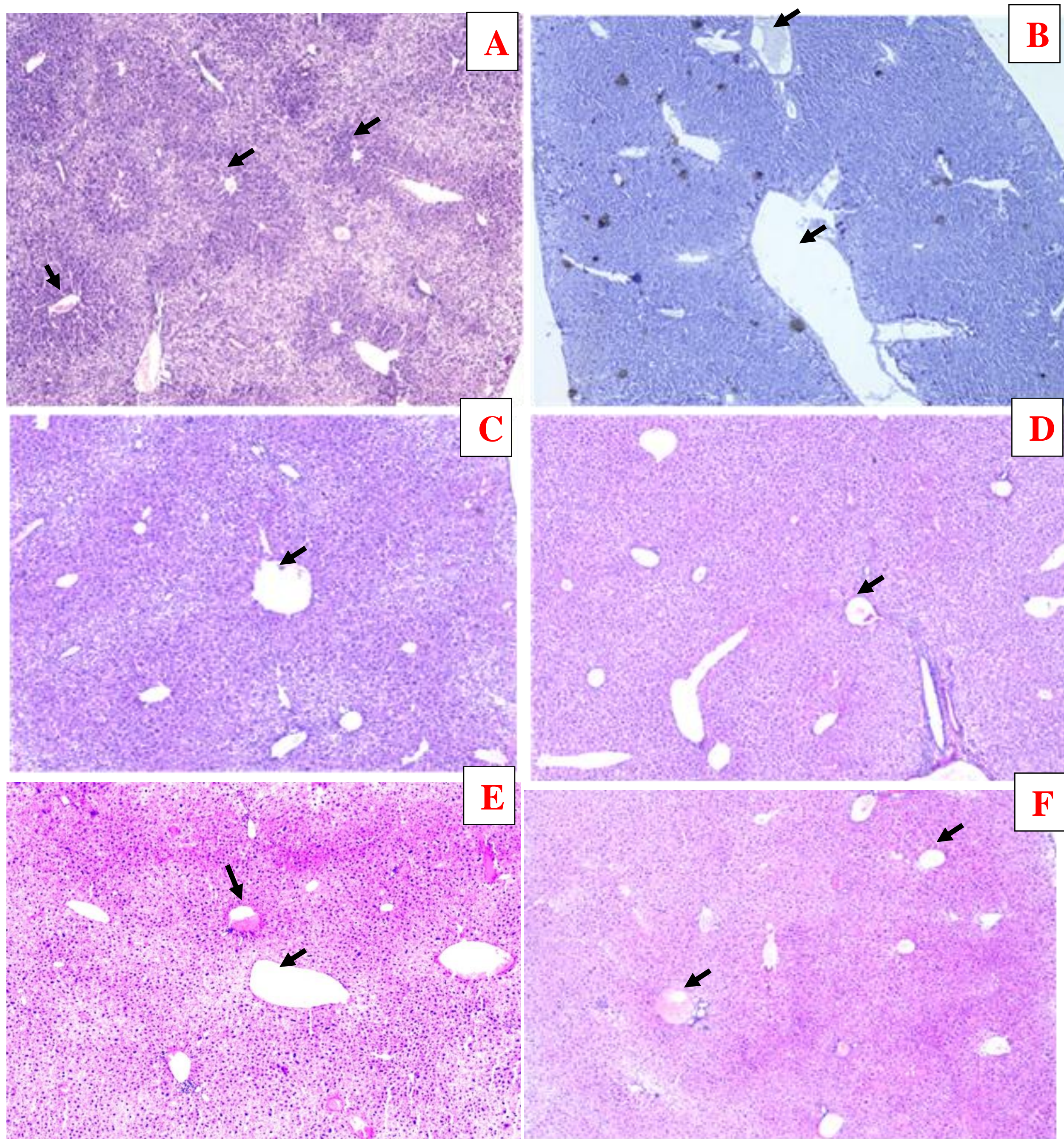


Fig. 1; Showing H&E staining of liver in different groups (40 x magnifications). (A) The control group showing normal architecture of hepatic central veins (↖). (B) The CP treated group showing super dilated and elongated hepatic central veins (↖). (C) The AP 20 mg/kg body weight treated liver showing central veins (↖) as controls. (D) Liver of mice treated with AP extract alone as 50 mg/kg body weight showing central vein (↖). (E) AP (20 mg /kg) extract on CP treated liver showing dilated central veins. (F) AP 50 mg/kg treatment reduced hepatic central vein dilations.

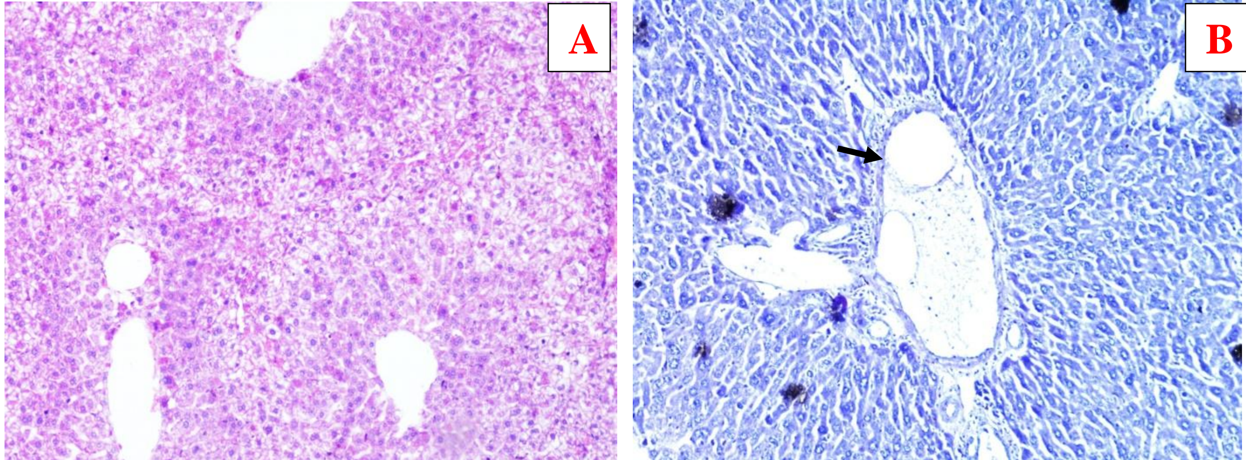
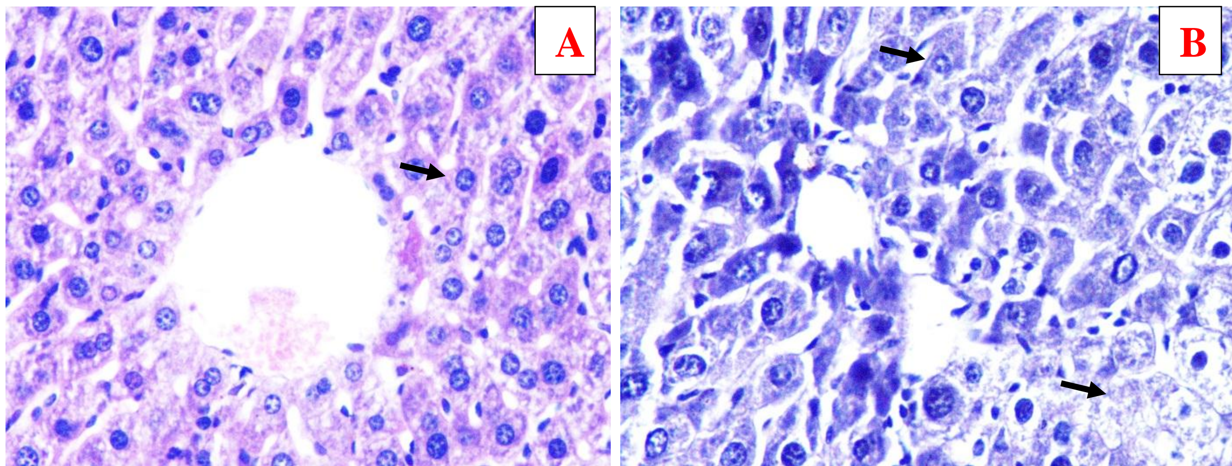
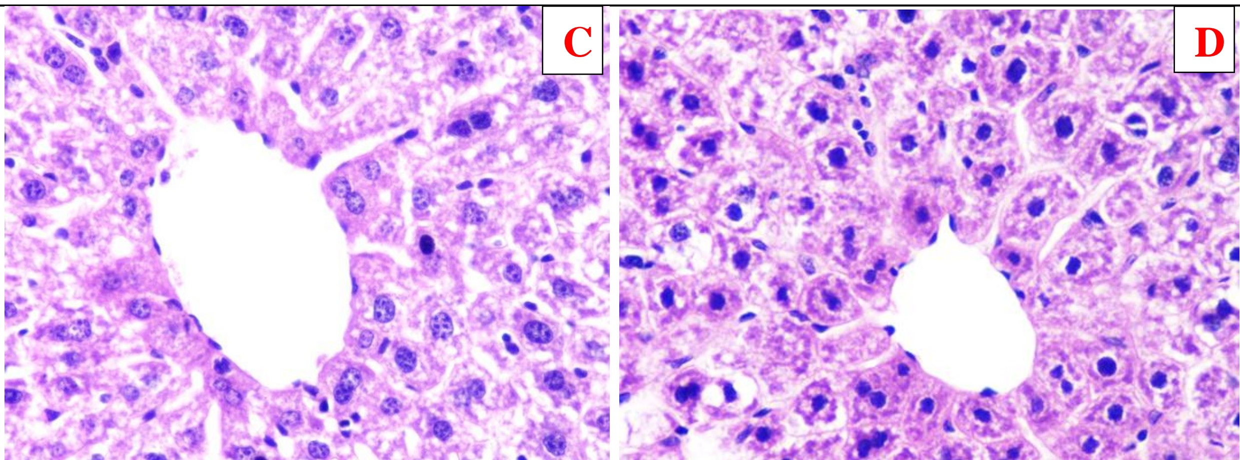


Fig. 2; Sections of liver (H&E x 100). (A) Control liver. (B) CP treated liver showing periportal round cells infiltration (↖).





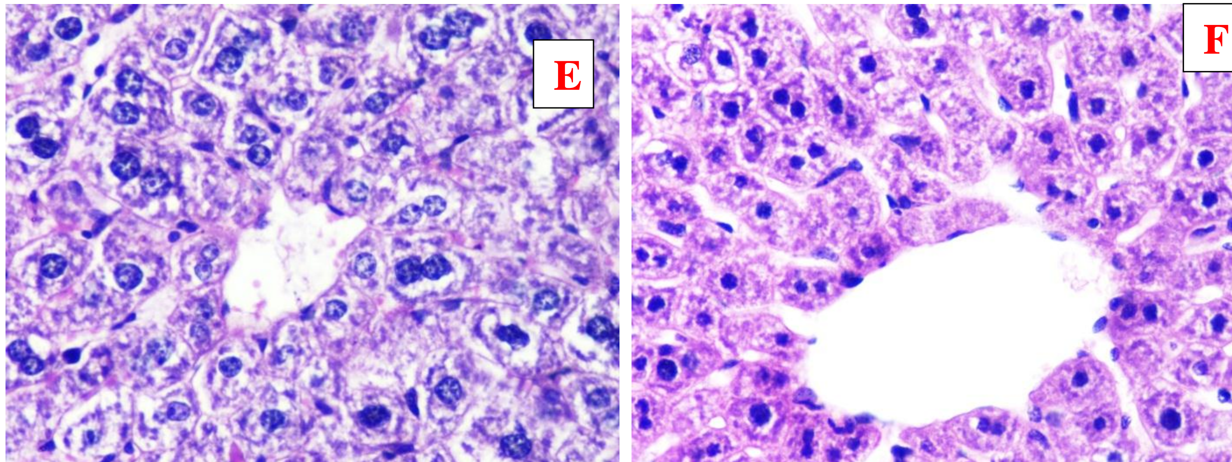


Fig. 3; Histological sections of liver (H&E x 400). (A) The control group showing normal architecture of hepatocytes (↖). (B) The CP treated group showing hepatic degeneration and necrotic lesions (↖). (C) The liver of mice received AP 20 mg/kg body weight alone was showing similar histopathology as controls. (D) Liver of mice treated with AP extract alone as 50 mg/kg body weight. (E) AP (20 mg /kg) extract did not show noticeable effect on CP induced hepatic damage, while (F) Extract of AP50 mg/kg showed reduced hepatic damage.

The most pronounced histopathological abnormalities were observed in the CP treated liver, where the normal arrangement of hepatic cord was disturbed and partial distortion in the architecture. Significant and prominent dilations in hepatic cords and sinusoids were observed (Fig.1B). Periportal round cells, including macrophages and lymphocytes are densely collected around the portal triad (Fig. 2B). Hepatocytes are showing plenty of degenerating and pyknotic nuclei. The density of hepatocytes is decreased and centrally located nuclei are fragmented. The intra cellular contents of the hepatocytes are extruded out and scattered here and there in the form of cellular debris which shows increased incidence of liver degenerations and apoptotic cell death (Fig. 3B). As a result of degeneration of hepatocytes, i.e. focal areas of patchy hepatic necrosis were seen. Inflammatory cell infiltrate can be seen in the liver sections (Fig. 3B).

The liver treated with low dose of AP (20 mg/ kg) along with CP did not prevent the CP induced toxicity. Degenerated, inflamed hepatocytes with frequent necrosis were still present (Fig. 1E & 2E). In contrast, high dose of AP treatment (50 mg/ kg) along with CP showed strong hepatoprotection by preventing the degeneration and necrosis (Fig. 1F & 3F). The cellular density was increased and the lumen of sinusoids was observed to be compressed in comparison to the controls. Kupffer cells were increased in number (Fig. 3F). The histological section of liver treated with AP extract (groups III and IV) only was comparable to the control (Fig. 3C & 3D). Liver sections showed normal hepatocytes, but at same time the dividing bi-nucleated cells were more frequent.

DISCUSSION

Cisplatin is one of the widely used drugs in cancer therapy, accumulates in the liver through blood. Its administration in vivo has known to cause several side effects including hepatotoxicity (6-8, 35). In the living systems, liver (mainly hepatocytes) is the first targeted

organ of different toxic chemicals and drugs including cisplatin which carries out more than 500 specialized metabolic activities and their detoxification (1,36). CP induced liver injury is mainly attributed to its toxic effect on hepatocytes by inhibiting their division and the oxidative stress. The present study showed the ameliorative effect of AP on degenerative changes in liver induced by cisplatin administration as AP is well known antioxidative and hepatoprotective agent.

In present study, single dose of CP (6mg /kg body weight) treatment showed dilatations of sinusoids, central vein and portal triad including smaller nuclei and fragmented chromatin with extruded cytoplasmic materials which at multifocal areas in haematoxylin and eosin stained sections. The findings were supported by a study done by Lu & Arthur (2006)(37). Irregular shaped, swollen hepatic lobules were also found in CP treated mice which was in accordance to the observations by Zicca *et al*. (2002)(38). Moreover in the liver sections many hepatocytes showed dark stained, intact pyknotic nuclei with perinuclear spaces which might be due to drug induced enhanced apoptosis. These results corroborate with the study of Kishimoto *et al.* (2000) who observed that cisplatin treatment results in histopathological abnormalities including the apoptosis (4). In a study by Ledda- Columbano *et al* (1991), it was observed that the presence of apoptosis may be the cause of hepatotoxicity which was preceded to necrosis by treatment of chemicals like thioacetamide [39]. In another study by Jie et al. (1998), it was stated that after cisplatin treatment, more apoptotic cells were observed in comparison to necrotic cells in the liver [7]. Necrotic cells having ruptured nuclear membrane and fragmented chromatin materials was also seen in our study. On the basis of these, it could be asserted that observed necrosis may be the continuation of enhanced apoptosis caused by CP induced hepatotoxicity. Weak eosinophilic stains in hepatocytes with inflammatory cells infiltration around sinusoids and central vein were also observed along with prominence of kupffer cells in hepatic sinusoids. Activation of kupffer cells have been reported [1] which might result in those degenerative changes as observed in the present study. When AP was administered along with CP the degenerative changes was reduced only at the high doses of AP (50 mg/kg). At low doses the AP was not able to reduce the degenerative changes as indicated by presence of degenerated and inflamed hepatocytes with frequent necrosis. But at high doses, the degeneration and necrosis of hepatocytes was much reduced and the cellular density was also increased. This showed the hepatoprotective role of AP against the toxicity induced by cisplatin was achieved at high doses of AP. The antioxidative and anti-inflammatory property of AP [21, 26, 29] might reduce the degenerative changes in liver.

CP act metabolically by activation of Cytochrome P 450 which activates hydroxylation reaction and forms free radicals such as superoxide, hydroxyl, singlet oxygen and hydrogen peroxide after reacting with molecular oxygen. These are reactive oxygen metabolites (ROM), act as a mediator of cellular enzymes inactivation, breakdown of DNA strands and lipid peroxidation (LPO) reaction in biological membranes. These elevated ROS level is thought to be responsible for various pathological conditions including metabolic disturbances in liver. Acting against the stress are antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) which act as a ROS scavengers. For the normal physiological processes, balance between oxidants and antioxidants are necessary but in stress, increased

ROS production declines the antioxidants level in liver, was may be the cause of hepatotoxicity [14, 40] as seen in our study. Because of antioxidant property of AP, its extract elevates the level of antioxidant which normalizes ROS level and lipid peroxidation (LPO) reaction and decreased the degenerative changes.

CONCLUSION

Single dose of CP produced the degenerative changes in liver by exerting its toxic effect. Natural agents such as AP which is rich in antioxidants was able to reduce the CP induced hepatotoxicity.

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