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HEPATOPROTECTIVE AND ANTIOXIDANT STUDY OF *ANDROGRAPHIS PANICULATA* AGAINST CYCLOPHOSPHAMIDE INDUCED HEPATOTOXICITY

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Abstract

The aim of the study was to assess the hepatoprotective potential of ethanolic extract of *Andrographis paniculata (*EAPE*)* against hepatotoxicity and liver injuries developed during cancer chemotherapy with cylophosphamide (CTX). Cyclophosphamide is a prodrug that undergoes hepatic metabolism to become the active 4-hydroxycyclophosphamide and toxic metabolites like phosphoramide mustard and acrolein. The toxic metabolites produced by CTX alter the hepatocellular membrane permeability by initiating lipid peroxidation and leading to hepatic injuries. The hepatoprotective activity of EAPE was assessed and compared with the ayurvedic preparation Liv. 52 (100 mg/kg) as well as the standard drug Silymarin (100 mg/kg). Six groups of Wistar rats (n = 6) were constituted. CTX intoxicated animals were treated with EAPE at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg of body weight. On the 14th day, blood samples were collected and examined for liver function parameters (SGOT, SGPT, ALP, and total bilirubin content). Liver sections were examined for histopathological transformations, and liver homogenates were further investigated for the antioxidant potential of EAPE against CTX induced oxidative stress. Supplementation with EAPE reported a significant ($p<0.001$) fall in SGOT, SGPT, ALP, and total bilirubin content. A significant ($p<0.001$) enhancement in the antioxidant effect of EAPE was also reported as a decrease in lipid peroxidation along with an increase in GSH, SOD level, and catalase activity. Histopathological healing in EAPE treated animals was evidenced by hepatic regeneration and the retention of normal hepatic architecture. This indicates that *A. paniculata* could be considered as an additional herbal supplement during cancer chemotherapy to avoid drug-induced hepatotoxicity.

Key words: *A. paniculata, anticancer drugs, anticancer, hepatotoxicity, sylimarin.*

Introduction

Drug-induced liver injury is a major adverse drug reaction observed during prolonged drug therapies or overdosing of drugs. The liver is the primary site for their metabolic transformation; therefore, cells of hepatic architecture are more vulnerably targeted by these drugs and their metabolites. Combating cancer with anticancer drugs, without harming normal, healthy cells is now a major challenge in both developed and developing countries. Cyclophosphamide (CTX) is an antineoplastic agent used in the management and treatment of Hodgkin lymphoma, non-Hodgkin lymphoma, multiple myeloma, leukemia, cutaneous T-cell lymphoma, and neuroblastoma. It is also recommended in combination with other anticancer agents to treat ovarian cancer, retinoblastoma, and cancer of the breast **(1).** In addition to this, cyclophosphamide is also prescribed as an immune suppressor to manage autoimmune disorders and during organ transplantations **(2).** Cyclophosphamide is a prodrug that, under extensive hepatic metabolism by Cyp-450, is converted into the therapeutically active metabolite 4-hydroxycyclophosphamide. 4-hydroxycyclophosphamide exists in tautomeric equilibrium with aldophosphamide. Some of the aldophosphamide was oxidized by hepatic aldehyde dehydrogenase (ALDH) into carboxycyclophosphamide and remained freely diffused into hepatocytes, where it was converted into phosphoramide mustard and acrolein[3]. Phosphamide alkylates the cross linking of the purine bases of DNA, producing immunosuppressive and antineoplastic effects, while acrolein causes hepatotoxicity by inducing oxidative stress. Hepatotoxicity is characterized as massive hepatic necrosis, hepatacellular injury with steatosis, and cholestasis **(4, 5, 6).** The plant has also been found to possess hepatoprotective, anti-inflammatory, and antioxidant properties, making it a promising candidate for the development of new drugs. Additionally, recent studies have shown that *A. paniculata* extracts may have potential in the treatment of certain types of cancer.

Andrographis paniculata (Burm.f.*)* Nees is an annual herbaceous medicinal plant belonging to the family Acanthaceae. In India, *A. paniculata* is commonly known as "Kalmegh". In Ayurveda, *A. paniculata is* included as a major herb in 26 different formulations used for the treatment of sour throat, viral infections, and fevers, as well as an antidote against snake bite poisoning **(7).**

Recently, it has been established that the diterpenoids of *A. paniculata* have hepatoprotective potential. Phytoconstituents of *A. paniculata*, andorgrapholide, and neoandrographolides are reported for their anti-inflammatory and hepatoprotective effects, while 14-deoxy-11, 12-di-dehydroandrographolide, and 14-deoxyabdrographolide are reported for their immune-stimulatory, anti-atherosclerotic, and hepatoprotective potentials **(8).** Phytoconstituents of *A. paniculata* process antioxidant effects by inducing cytochrome P450 enzymes and modulating the content of glutathione (**9**). Additionally, *A. paniculata* has been traditionally used in Ayurvedic medicine for treating respiratory infections, fever, and digestive disorders due to its antibacterial and antiviral properties. Its potential as an alternative treatment for various diseases is currently being explored through clinical trials.

A literature survey reveals that no pharmacological studies have been conducted to this date to evaluate the hepatoprotective potential of *A. paniculata* against cyclophosphamide induced hepatotoxicity. Accordingly, the present investigation is designed to assess the healing potential of an ethanolic extract of *A. paniculata* (EAPE) on cyclophosphamide induced liver injuries. The study comprises the estimation of liver function parameters such as serum enzymes (SGPT, SGOT, ALP, and total bilirubin

content). The antioxidant potential of the EAPE against oxidative stress developed by cyclophosphamide was evaluated with the estimation of levels of MDA, SOD, reduced glutathione, and catalase activity in liver tissue homogenates. Liver sections were examined to assess the healing potential of the EAPE against the histopathological transformation caused by cyclophosphamide.

Methodology

Chemicals

Cyclophosphamide and silymarin were procured form Sigma-Aldrich. All other chemicals, solvents, and reagents used were of analytical grade.

Collection of plant material

The whole plant of *A. paniculata* was collected from the local region of Agra, Uttar Pradesh. The Regional Research Institute, Jhansi, identified and authenticated the *A. paniculata* specimen (RARI-JHS/1782-28680). The whole plant was collected, dried under shade at room temperature, and pulverized by a mechanical grinder to a coarse powder.

The cold maceration method was preferred for the extraction to avoid the deterioration of the phytoconstituents. The coarse powder (500 g) of the whole plant of *A. paniculata is* successively macerated with petroleum ether (5 lit.) and ethanol (5 lit.). The extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C until further use. The yield of each extract was recorded and the extracts were evaluated for their phytochemical composition and biological activities.

Phytochemical screening

The ethanolic extract was screened for the presence of different phytoconstituents such as carbohydrates, alkaloids, glycosides, terpenoids, steroids, tannin, flavonoids, and phenolic compounds **(10).** The extract was also estimated for its total phenolic and total flavonoid contents.

Pharmacological Studies

Animals

The present studies were carried out on Wistar rats weighing 210±10g of either sex and procured from the PBRI animal house. The animals were housed under standard conditions of humidity, temperature (25±2 ◦C) and light (12 h light/dark). They were fed a standard rat pellet diet and water ad libitum. Animal based experimental studies were conducted as per the ethical guidelines of the Institutional Animal Ethics Committee (Reg. No. 1824/PO/RC/S/15/CPCSEA).

Acute toxicity study of EAPE

An acute oral toxicity study of an extract of EAPE was performed as per the OECD-423 guidelines. The extract was administered orally at four dose levels (5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg) and observed for toxic symptoms, body weight changes, and lethality. Results are summarized in Table 1 **(11).**

Hepatoprotective studies of EAPE against cyclophosphamide-induced hepatotoxicity

The hepatoprotective activity of the EAPE was evaluated against cyclophosphamide induced toxicity. Since the hepatoprotective potential of *A. paniculata* has already been established, no animal control group was created. The Wister strain of albino rats weighing 200 g was selected and randomly divided into 6 groups (n = 6 animals) as follows:

No drug control animal group was previously considered for the hepatoprotective potential of *A. paniculata* previously reported.

Group I: Control treated with normal saline

Group II: Only 200 mg/kg cyclophosphamide was introduced i.p. on the first day.

Group III: 200 mg/kg cyclophosphamide in a single dose introduced i.p. on the 1st day, and 250 mg/kg EAPE orally continued for 14 days.

Group IV: 200 mg/kg cyclophosphamide in a single dose introduced i.p. on the 1st day + 500 mg/kg EAPE orally continued for 14 days.

Group V: 200 mg/kg cyclophosphamide in a single dose introduced i.p. on the 1st day + 1000 mg/kg EAPE orally continued for 14 days.

Group VI: 200 mg/kg Cyclophosphamide single dose introduced i.p. on the 1st day + 100 mg/kg Liv.52 (Ayurvedic preparation) orally continued for 14 days.

Group VII: 200 mg/kg Cyclophosphamide single dose introduced i.p. on the 1st day + 100 mg/kg Silymarin orally continued for 14 days.

Serum preparation for estimation of the liver functional parameters

After 24 hours of final administration on the 14th day of studies, the blood was taken from the microcentrifuge retro-orbital sinuses of the experimental animals and collected in Eppendorf microcentrifuge tubes and immediately in a cooling microcentrifuge apparatus at 7000 rpm at 4ºC for 15 minutes to obtain clear serum. The resultant serum was transferred into fresh sterilized Eppendorf microcentrifuge tubes and estimated for the serum biochemical markers (SGPT, SGOT, ALP, and total bilirubin content).

Antioxidant studies of EAPE

In the present study, the oxidative stress generated by cyclophosphamide and its numerous metabolites was assessed after 24 hours of the final administration on the 14th day of the study. The animals were sacrificed, and their liver tissue was isolated and washed with ice-cold physiological saline to remove the blood. Liver were excised and homogenized in 0.1M tris-HCl buffer (pH 7.4). The homogenate was centrifuged to remove the cell debris, unbroken cells, nuclei, erythrocytes, and mitochondria. The supernatant aliquots were estimated for the tissue enzymatic antioxidant assays for superoxide dismutase (SOD), catalase activity, lipid peroxidase, and glutathione reductase (GSH) **(12).**

Estimation of SOD

The oxidative stress marker superoxide dismutase estimation is based on the principle of generation of the superoxide radicals by the NADH-phenazine methosulfate (PMS) system, which causes the reduction of tetrazolium salts nitro blue tetrazolium (NBT) into blue formazan, which is further measured spectrophotometrically at 560nm against a blank. The SOD in the samples competes for the generated superoxide radical, thereby inhibiting the reaction of tetrazolium reduction **(12).** SOD activity was determined from the ability of the tissue homogenate to scavenge the superoxide anion generated by cyclophosphamide induced toxicities, as per the method designed by Kakkar et al. (1984). One unit of SOD enzyme activity is represented as the enzyme concentration needed to inhibit the optical density at 560nm of chromogen production by 50% in one minute and expressed as specific activity in milliunits/mg protein **(13).**

Estimation of lipid peroxidation

The estimation of lipid peroxidation was conducted to assess the ROS-mediated damage of the cell membranes of the hepatocytes. This has been attributed to the fact that under oxidative stress, peroxidation of the polyunsaturated fatty acids produces malondialdehyde (MDA) as an end product **(12).** Nur Alam et al. (2013) The level of the generated MDA was measured on reaction with thiobarbituric acid (TBARS) in acidic medium at 100 ºC to develop a pink-red colour product, which was extracted with butanol: pyridine (15:1), and its absorbance was measured at 520–535 nm spectrophotometrically **(12, 14).**

Catalase Activity

The estimation of catalase activity is based on the principle of decomposition of hydrogen peroxide into water and oxygen in the presence of $K_2Cr_2O_7$ and an acetic

acid reagent *(Beers and Sizer, 1952).* The enzymatic activity of the catalase was measured colorimetrically at 610nm as the disappearance of hydrogen peroxide. Each unit represented the amount that degrades 1 umol of hydrogen peroxide per minute **(12, 15).**

Estimation of reduced GSH

Glutathione, a low molecular weight thiol, participates in the metabolic protective functions of reduction of hydroperoxide, detoxification of xenobiotics, and scavenging of the generated free radicals. In 1959, G. L. Ellman came up with a way to measure the amount of GSH based on the ability of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman reagent) to react with compounds with sulfhydryl groups to make disulfide (GS-TNB) and 2-nitro-5-thiobenzoic acid (TNB). The level of the TNB is quantified spectrophotometrically by measuring the absorbance of the anion (TNB^{-2}) at 412nm against a blank. Absorbance values were compared with a standard curve generated from known GSH **(12).**

Histopathological investigation

The liver tissues were immersed in a 10% formalin solution for histopathological examination. These tissues were processed, dehydrated in different grades of alcohol, cleared in toluene, and impregnated in molten paraffin wax for specified periods. Processed tissues were embedded in fresh molten paraffin wax and allowed to set. Sections were microtomed at 5µm thickness and stained with hematoxylin-eosin (H&E). Stained sections were examined microscopically at 10X objective for pathological findings.

Statistical Analysis

Values are expressed as MEAN±SD at n=6. Results analyzed statistically using a one-way ANOVA followed by a Bonferroni t-test. Significant level represented as *, ($p<0.05$); **, ($p<0.01$); ***, ($p<0.001$), ns-no significant difference.

Result and Discussion

The whole plant of *A. paniculata* was macerated and extracted in ethanol. The percentage yield of extract in the ethanolic solvent system obtained was 3.02%. A qualitative test for phytochemicals confirmed the presence of alkaloids, saponins, tannins, glycosides, terpenoids, steroids, flavonoids, and phenolic compounds. Quantitative estimations for total phenolic content (86.50 mg/gm equivalent to gallic acid) and total flavonoid content (68.50 mg/gm equivalent to rutin) were reported.

Acute toxicity assessment of EAPE

Acute toxicity studies were conducted to assess the safety aspects of EAPE. Neither any mortality nor any symptoms of toxicity up to the dose of 2000 mg/kg were reported during the 72-hour observation of animals in all groups. This complies with the previous results of *A. paniculata*, which signifies the safety of EAPE up to a dose of 2000 mg/kg **(16).**

Table 1: Acute toxicity studies of EAPE.

Group	Dose (mg/kg)	Rat No.	Day of Death	Body Weight (gm)			No. Death/Tested
				0 Day	7 Day	14 Day	
A	5 mg/kg	R1	--	191.51	193.22	195.50	0/3
	"	R2	--	192.45	193.86	195.44	
	"	R3	--	196.76	197.82	199.22	
B	50 mg/kg	R1	--	205.28	206.55	207.99	0/3
	"	R2	--	209.50	301.84	303.62	
	"	R3	--	203.72	205.48	207.69	
C	300 mg/kg	R1	--	206.22	208.39	210.77	0/3
	"	R2	--	201.87	203.69	205.82	
	"	R3	--	201.71	202.98	204.66	
D	2000 mg/kg	R1	--	202.69	204.52	206.17	0/3
	"	R2	--	207.11	209.39	211.77	
	"	R3	--	208.65	210.43	212.71	

Evaluation of the hepatoprotective potential of EAPE

Hepatotoxicity is a major side effect of cyclophosphamide. Cyclophosphamide undergoes extensive hepatic metabolism by the Cyp-450 mixed function oxidase system to produce active metabolites, including 4-hydroxycyclophosphamide, phosphoramide metabolites, and acrolein **(17).** Cyclophosphamide and its metabolites distorted hepatic cell membrane integrity by initiating oxidative degradation of lipids in cell membranes, resulting in hepatic injuries with leaching out of the hepatocellular contents and enzymes such as SGOT, SGPT, and ALP, as well as bilirubin, into systemic circulation **(17).**

The results of serum biochemical parameters for group II animals (treated with cyclophosphamide) reported a remarkable elevation in SGOT, SGPT, and ALP. This indicates intense hepatic injuries produced by cyclophosphamide. Elevation of AST and ALP is associated with hepatic necrosis and alteration of hepatic membrane permeability, causing leakage of enzymes into blood circulation. Animals treated with EAPE have reported a significant fall in enzyme levels (SGOT, SGPT, and ALP) (table 2) in a dose dependent manner; this signifies that *A. paniculata* has hepatic wound healing potential. Supplementation with EAPE at a dose of 250 mg/kg decreases the elevated level of enzymes by up to 50% while restoring them to normal at a dose of 1000mg/kg paniculata/kg. The hepatic healing effect of *A. paniculata* at a dose of 1000 mg/kg is fairly similar to the effect produced by the pure standard drug silymarin. This signifies that 250 mg/kg could be considered a minimum dose for the significant hepatoprotective effect of *A. paniculata.*

Increase in Bilirubin content indicates the incidence of hepatic necrosis and its accumulation, characterized as hepatocyte functional insufficiency, biliary insufficiency, or an increase in hemolysis. Hepatic necrosis, obstruction of the hepatic

venous flow, and jaundice are all symptoms of severe sinusoidal obstruction syndrome, which has also been associated with cyclophosphamide administration (**18, 19**). Raised serum levels of bilirubin indicate the prevalence of jaundice. Cyclophosphamide increased the total bilirubin from 0.3 $\pm$ 0.03 in the control group to 2.11$\pm$0.19 (mg/100 ml of serum); this indicates that cyclophosphamide alters the metabolism of the bilirubin. Treatment with *EAPE* re-establishes the serum bilirubin level in a dose dependent manner, and at a dose of 1000 mg/kg, the serum bilirubin level is fairly near normal.

Table 2: Evaluation of the Hepatoprotective Potential of the EAPE.

Group No.	Treatments	Liver Functional Parameter						
		Body weight (gm)	Absolute liver wt. (gm)	Relative liver wt. (gm/100)	AST (IU/dL)	ALT (IU/dL)	ALP (IU/dL)	Bilirubin (mg/dL)
I	Vehicle only	214±1.41	5.17±1.17	2.42±0.56	27.4±5.45	25.64±5.46	72.49±7.35	0.3±0.03
II	CTX + Vehicle	211.17±2.32	8.5±1.05	4.02±0.45	113.87±3.28	91.05±3.31	236.83±5.19	2.11±0.19
III	CTX + AP 250mg/kg	206.33±6.77 ^{ns}	6.17±1.33**	2.98±0.56**	79.85±4.1**	61±3.31**	177.64±5.07**	1.87±0.24**
IV	CTX + AP 500mg/kg	201.42±3.45**	5.17±1.17**	2.56±0.53**	57.75±8.42**	42.14±4.93**	128.37±13.5**	0.71±0.19**
V	CTX + AP 1000mg/kg	203.67±4.03^{ns}	5.12±0.47**	2.51±0.19**	49.8±5.05**	39.19±5.41**	123.4±5.07**	0.56±0.21**
VI	CTX+Liv.52,100mg/kg	201.13±3.33**	4.67±0.55**	2.32±0.26**	38.1±3.4**	32.39±3.49**	74.58±10.68**	0.35±0.09**
VII	CTX+ SIL 100mg/kg	203.22±3.01 ^{ns}	4.5±0.55**	2.21±0.24**	37.13±4.18**	30.04±6.2**	65.25±7.01**	0.29±0.15**

Values are expressed as MEAN±SD at n=6. Results analyzed statistically using One-way ANOVA followed by Bonferroni t-test. Significant level**s are** represented as *P<0.050, **P<0.001 and ^{ns}P>0.001 (ns-no significant difference) compared to the control. EAPE, ethanolic extract of *A. Paniculata* CTX, Cyclophosphamide (200 mg/kg); SIL, Silymarin (100 mg/kg); SGOT, Serum glutamic oxaloacetic transaminase (per min per mg protein); SGPT, Serum glutamic pyruvic transaminase, (per min per mg protein); ALP, alkaline phosphatase (one king Armstrong unit 1 UI-1); Bilirubin, gm/dL.

Evaluation of oxidative stress

Oxidative stress is a state characterized by the elevation of intracellular ROS (reactive oxygen species). Reactive oxygen species (ROS) are the common by-products of chemical molecules containing oxygen that further break down to form free radicals. ROS include superoxide anion (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). Indeed, the hepatic cellular metabolisms are engaged to generate these ROS, but under oxidative stress, excessive free radicals are generated, which compromise hepatic cellular health and contribute to liver cirrhosis by inducing the destruction of DNA, proteins, and lipids through peroxidation. The liver has a classical antioxidant enzyme defense system made up of superoxide dismutase (SOD), which gets rid of superoxide anions and reduces the harmful effects of free radicals; glutathione reductase (GS), which is important for keeping and regaining reduced levels of glutathione in the cytoplasm; lipid peroxidase; and catalase, which breaks down H_2O_2 and protects the tissue from highly reactive hydroxyl radicals. An imbalance between the ROS production and antioxidant defense mechanisms of the liver may lead to hepatic injuries. Oxidative stress biomarkers are therefore important tools to assess hepatic cellular health.

It has been established that extensive hepatic metabolism of cyclophosphamide into acroline leads to hepatocellular toxicity. Cyclophosphamide toxicity decreases the levels of SOD, GSH, and

CAT below normal; this indicates the over accumulation of free radicals **(16).** Results of oxidative stress studies, shown in Table 3, reveal that cyclophosphamide causes excess lipid peroxidation, with a 3-fold amplification in the level of malondialdehyde (MDA), along with a fall in the level of the SOD, GSH; as well as a declination in the CAT activity when compared with the control group **(20).** Treatment with EAPE extract restored the raised levels of SOD, GSH, and CAT activity in a dose dependent manner, and at a dose of 1000 mg/kg, the level reached near normal. This signifies the ability of *A. paniculata* to nullify cyclophosphamide toxicity by establishing a balance between the hepatic antioxidant enzyme defense system and the production of ROS.

Table 3: Antioxidant Study of EAPE.

Group No.	Group	Parameter			
		LPO	SOD	GSH	CAT
I	Vehicle only	15.58±1.85	55.59±6.01	2.41±0.04	38.09±4.89
II	CTX + Vehicle	50.78±2.05	10.57±3.53	0.41±0.02	12.42±0.81
III	CTX +Sample EAPE 250 mg/kg	36.47±1.98**	23.05±2.28**	1.16±0.01**	16.98±1.85 ns
IV	CTX +Sample EAPE 500 mg/kg	22.22±1.39**	29.42±5.13**	1.21±0.01**	22.43±2.86**
V	CTX +Sample EAPE 1000 mg/kg	17.11±1.02**	36.2±2.85**	1.42±0.01**	26.26±4.45**
VI	CTX+ Liv.52 100 mg/kg	15.72±0.99**	50.7±3.36**	1.91±0.08**	34.74±3.65**
VII	CTX+ SIL 100 mg/kg	15.69±1.82**	50.16±5.41**	2.09±0.04**	36.65±2.86**

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test. Significant levels are represented as *P<0.050, **P<0.001 and ^{ns}P>0.001 compared to the control. EAPE, ethanolic extract of *A. Paniculata*; CTX, cyclophosphamide; SIL, Silamyrin; LPO, Lipid peroxidise (nmol MDA/mg tissue); SOD, superoxide dismutase (units/min/mg protein); GSH, Glutathione reductase (1µM of NADPH/min); CAT, Catalase (µm H_2O_2 consumed/min/mg protein).

Histopathology examination of EAPE

Hepatic histopathology was examined to evaluate the hepatoprotective effect of *EAPE* against cyclophosphamide induced toxicity. The liver sections of animals in Group I showed normal hepatic architecture with radially arranged hepatic cords around the central vein. Animals receiving a single dose of 200 mg/kg of cyclophosphamide (Group II) developed severe hepatic toxicity, evidenced by severe necrosis, hepatic congestion, and inflammation. There were also indications of intense granulocytic infiltration. Liver sections of animals treated with 250 mg/kg of EAPE (Group III) reveal a partial healing effect with the initiation of regeneration and restoration of the normal 500 mg parenchyma. Animals kept on treatment with 500 mg/kg of EAPA (Group IV) reveal remarkable regeneration and restoration of hepatic architecture. Liver sections of animals treated with 1000 mg/kg of EAPE (Group-V) showed a retention of the normal hepatic architecture with regular hepatocytes cords and sinusoidal arrangement, and the animals treated with Silymarin (100 mg/kg) showed normal heaptic architecture **(21).**

Liver section of Group I

Histology of the liver sections of Group I animals shown in Figure 1 reveals normal hepatocytes with well-preserved cytoplasm, a prominent nucleus, and radially arranged hepatic cords surrounding the central vein.

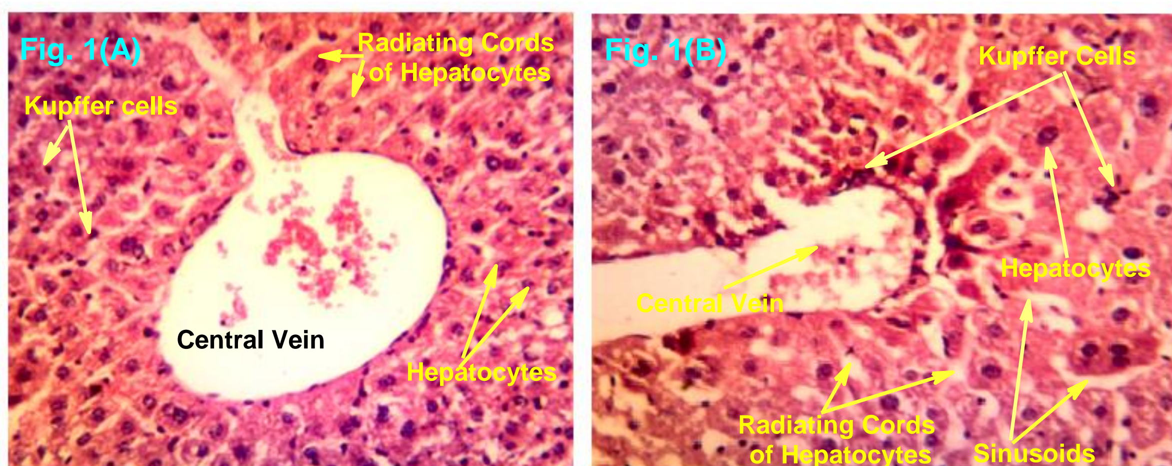


Figure **1:** Liver sections of animals belong to Group I (Control).

Liver section of Group II

Animals on normal saline (Group II) showed severe hepatic injuries in their liver sections, including necrosis, congestion, and inflammation. The image also depicts cholangitis, which is characterized by a severe mononuclear cell infiltration in the portal tract and rupturing of the hepatic cords. Intense bile duct cholangitis and inflammation with hemorrhagic clotting were visible in Figs. 2 (A) and 2 (B).

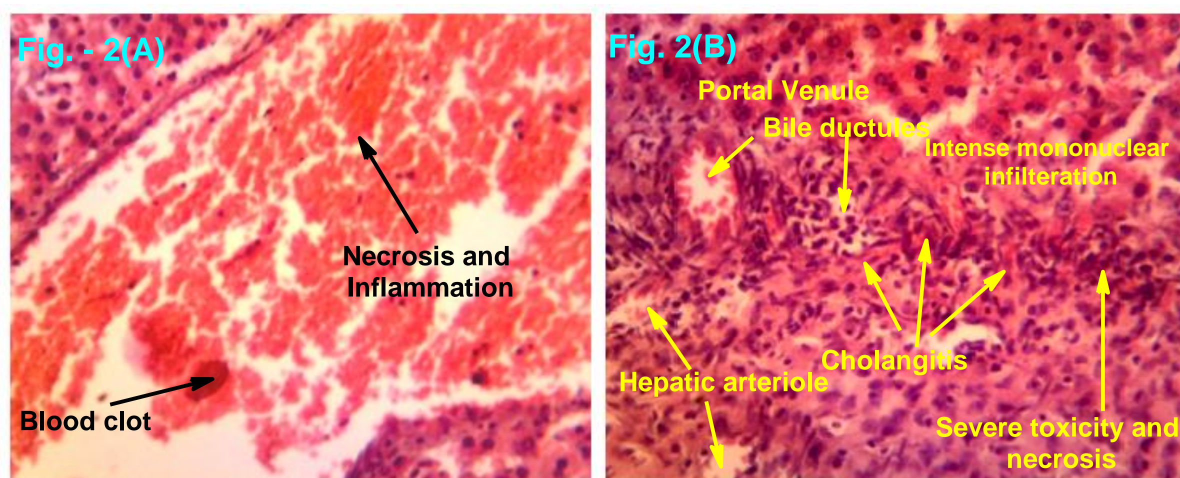
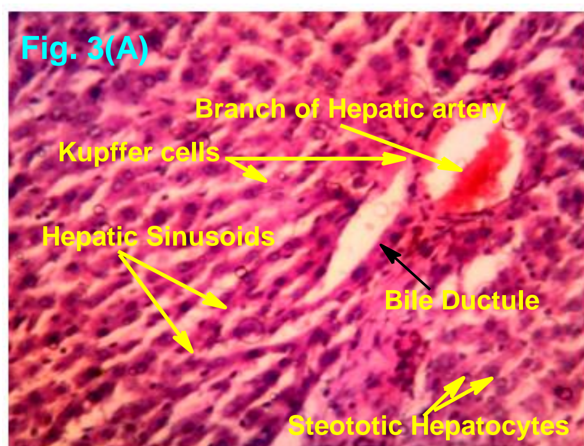
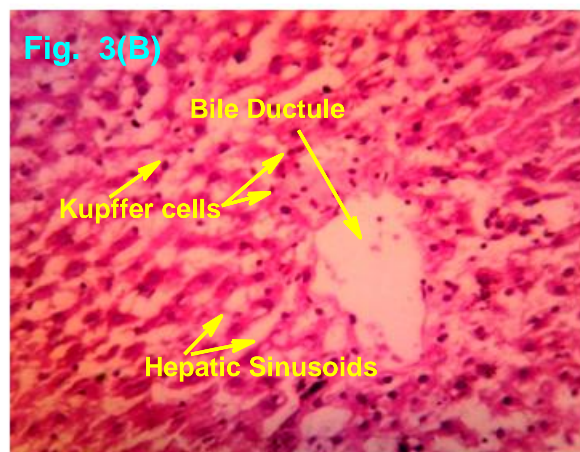


Figure 2: Liver sections of animals belonging to Group II supplemented only with normal saline

Liver section of Group III

Liver sections from animals given 500 mg/kg of AC extract displayed abundant hepatocytes in a dense hepatic parenchyma. Hepatic architecture is restored and there is no centrilobular congestion, steatosis, or granulocytic infiltration in either Fig. 4(A) or 4(B).



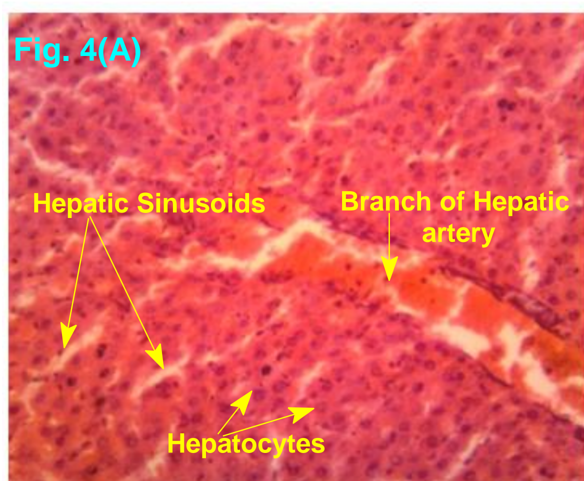


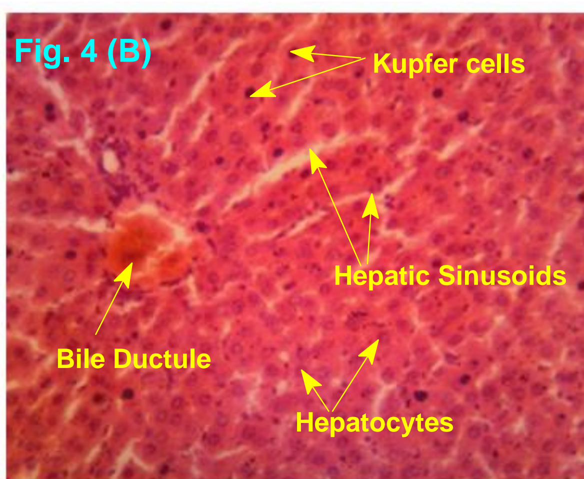
Group III (Cyp 200mg/kg + *A. paniculata* 250mg/kg)

Figure 3: Liver sections of animals belong to Group III treated with 250mg/kg of AP extract

Liver section of Group IV

Animal liver sections treated with 500 mg/kg of AC extract showed a dense hepatic parenchyma with copious hepatocytes. Fig 4(A) & 4(B) reflect restoration of hepatic architecture and also lack centrilobular congestion, steatotic hepatocytes, and an incidence of granulocytic infiltration.



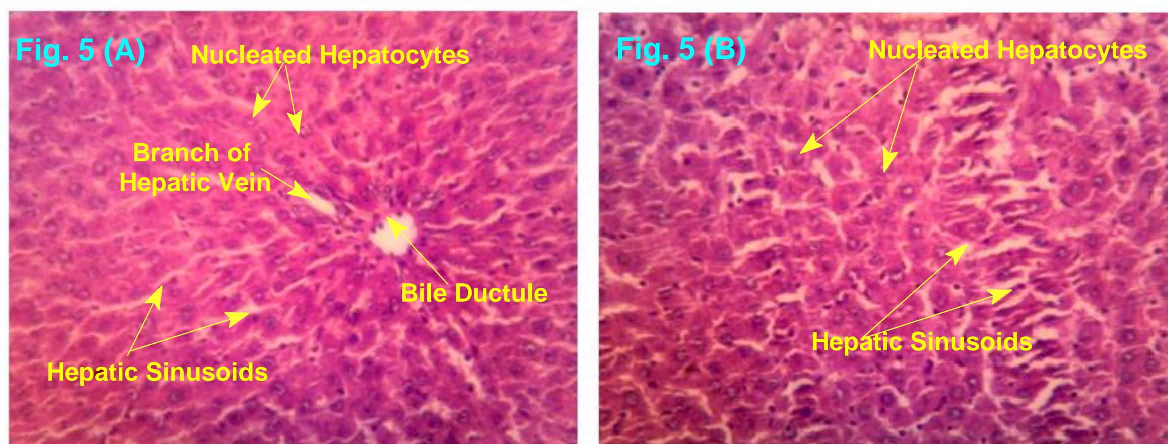


Group IV (Cyp 200mg/kg + *A. paniculata* 500mg/kg)

Figure 4: Liver sections of animals belong to Group IV, treated with 500 mg/kg of AC extract

Liver section of Group V

In contrast, liver sections of Group V treated with 1000 mg/kg of AP extract showed a significant reduction in hepatic parenchymal distortion and granulocytic infiltration. Fig 5(A) and 5(B) show dense hepatic parenchyma with nucleated heaptocytes; lacking cellular congestion and an incidence of granulocytic infiltration.This suggests that AP extract may have a protective effect on liver tissue. Further studies are needed to determine the mechanism of action and potential therapeutic applications of AP extract in liver diseases.

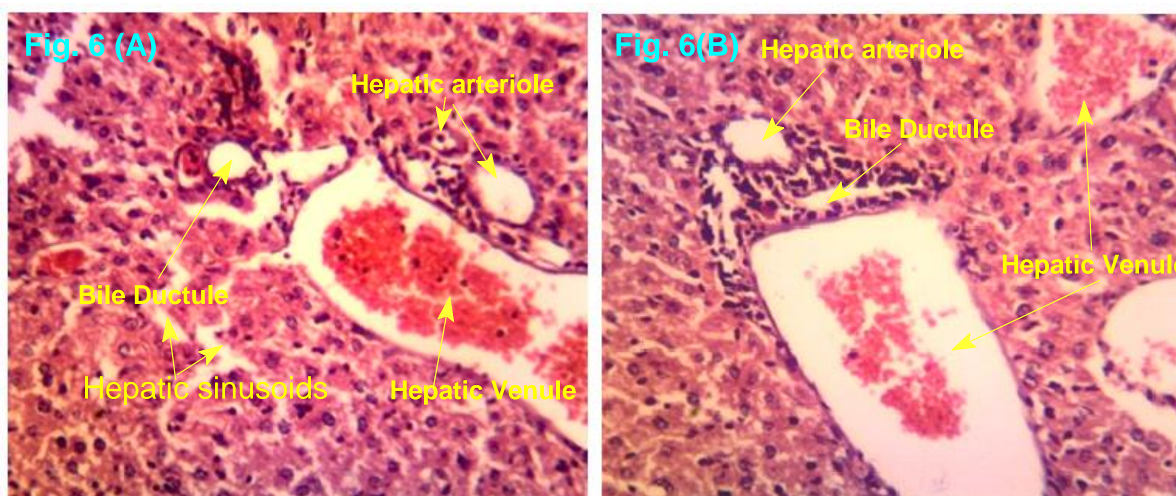


Group V (Cyp 200mg/kg + *A. paniculata* 1000mg/kg)

Figure 5: Liver sections of animals belonging to Group V treated with 1000 mg/kg of AP extract

Liver section of Group VI

Modest deformation of the hepatic parenchyma with granulocytic accumulation was observed in animal liver sections treated with 100 mg/kg of Liv 52. The hepatic parenchyma in Figs. 6(A) and 6(B) is loose and distorted, and there are mild granulocytic infiltration and steatotic hepatocytes.



Group VI (Cyp 200mg/kg + Liv-52 100mg/kg

Figure 6: Liver sections of animals belonging to group VI treated with 100 mg/kg of Liv 52

Liver section of Group VII

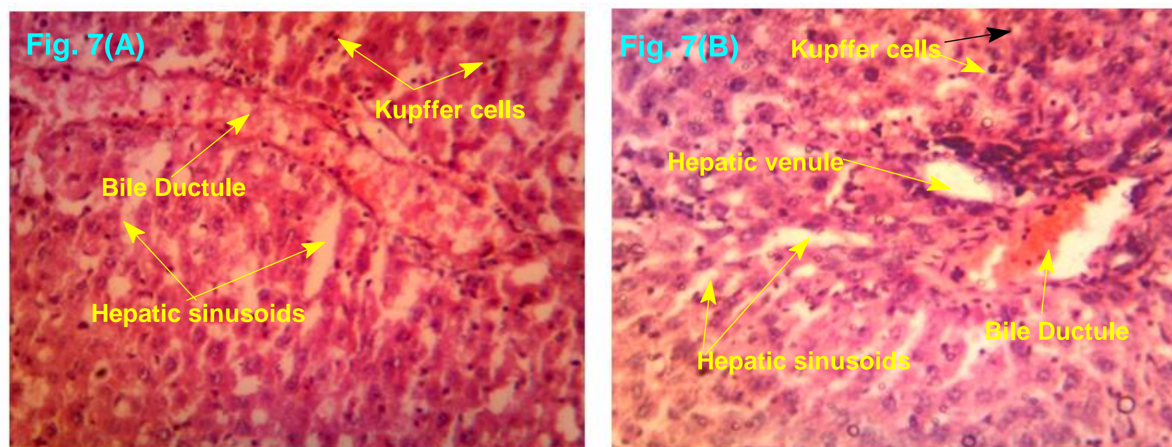


Figure 7: Liver sections of animals belonging to group VII treated with 100 mg/kg Siylmarin

Conclusions

The results of the present study reveal that CTX exposure elevates the level of serum liver functional enzymes and develops oxidative stress along with distortion of hepatic architecture. Supplementation with *A. paniculata* restores the level of serum liver functional enzymes by preventing LPO and reversing oxidative stress by enhancing the antioxidant system. Based on the results of the present study, *A. paniculata* could be considered as a supplement during CTX chemotherapy to minimize hepatic toxicities and also as a promising natural alternative for protecting the liver from drug-induced toxicity.

However, further studies are needed for the standardization of *A. paniculata* extract and the identification and isolation of the phytoconstituents for its hepatoprotective activity. Indeed, a study will also be required to explore the exact mechanism necessary to establish the hepatoprotective potential of *A. paniculata*.

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Conflict of thanks to the

The author declares that there is no conflict of interest.

Author(s) Contribution

The conceptualization and formal analysis of the work were planned and conducted by the PM. The work was conducted and completed under the supervision of the SM and SKP. The writing and original drafting of the manuscript were compiled by SKM. The manuscript was finally reviewed and edited by the SKP.

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