Available online on www.ijppr.com

International Journal of Pharmacognosy and Phytochemical Research 2022; 14(4);37-44

ISSN: 0975-4873

Research Article

Assessment of Healing Prospective of *A. Paniculata* on Cyclophosphamide Induced Liver Injuries

Paliwal Sarvesh Kumar[1], FaujdarSamriti[1], Mishra Sunil Kumar[2] Mishra Pratibha[1]

[1] Department of Pharmacy, BanasthaliVidyapith, Rajasthan, India
[2]Department of Pharmacy, S N Medical College, Agra

Received:30th Sept. 22; Revised:19th October 22, Accepted:25th Nov., 22; Available Online:25th Dece., 22

ABSTRACT

Cyclophosphamide (CPP) is an immunosuppressive agent, widely prescribed in cancer therapy and in management of autoimmune disorders. Cyclophosphamide is a prodrug on hepatic metabolism converted into active 4-hydroxycyclophosphamide and toxic metabolites such as phosphoramide mustard and acrolein. The produced toxic metabolites of CPP alter the hepatocellular membrane permeability by initiating lipid peroxidation and leads hepatic injuries. Present study was conducted to establish the healing prospective of ethanolic extract *A. paniculata* (EAPE) on cyclophosphamide induced liver injuries in Wister rats. Six groups of Wistar rats (n=6) were constituted. Intoxicated animals were treated with EAPE at dose of 250mg/kg, 500mg/kg and 1000mg/kg. On 14th day, blood samples were collected and examined for liver function parameters (SGOT, SGPT, ALP and total bilirubin content). Livers were examined for histopathological transformation and investigated for oxidative stress. Supplementation with EAPE reported a significant ($p< 0.001$) fall in SGOT, SGPT, ALP as well as in total bilirubin content. A significant ($p<0.001$) enhancement in antioxidant effect of EAPE were also reported as decrease in lipid peroxidation along with increase in GSH, SOD level and catalase activity. Histopathological investigations evidenced with healing and retention of normal hepatic architecture.

Keywords: *A. paniculata,* Cyclophosphamide*,* Hepatotoxicity, Phosphoramide.

INTRODUCTION

Cyclophosphamide (CPP) is an antineoplastic agent, used in the management and treatment of Hodgkin lymphoma, Non-Hodgkin lymphoma, multiple myeloma, leukaemia, cutaneous T-cell lymphoma, and neuroblastoma. It is also recommended in combination with other anticancer agents to treat ovarian cancer, retinoblastoma and cancer of breast[1].In addition to this cyclophosphamide also prescribed as immune suppressor to manage autoimmune disorders and during organ transplantations[2]. Cyclophosphamide is a prodrug, under extensive hepatic metabolism by Cyp-450 converted into therapeutically active metabolite 4-hydroxycyclophosphamide. 4-hydroxycyclophosphamide exists in tautomeric equilibrium with aldophosphamide. Some portion of the aldophosphamide oxidised by hepatic aldehyde dehydrogenase (ALDH) into carboxy cyclophosphamide and remain were freely diffuses into hepatocytes and converted into phosphoramide mustard and acrolein[3]. Phosphramide alkylate the cross linking of the purine bases of DNA produces immunosuppressive and antineoplastic effect, while acrolein causes hepatotoxicity by inducing oxidative stress. Hepatotoxicity characterised as massive hepatic necrosis, hepatacellular injury with steatosis, and cholestasis[4,6, 5].

Andrographispaniculata (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* included as major herb in 26 different formulations used for the treatment of sour throat, viral infections and fever as well as an antidote against snake bite poisoning[7].

Recently this has been established that the diterpenoids of the A Paniculata have hepatoprotective potential. Phytoconstituents of A. Paniculata, Andorgrapholide and Neoandrographolides are reported for their anti-inflammatory and hepatoprotective effect, while 14-deoxy-11, 12-di-dehydroandrographolide and 14-deoxyabdrographolide reported for their immune-stimulatory, anti-atherosclerotic, and hepatoprotective potentials[8]. Phytoconstituents of *A. paniculata* process antioxidant effect by inducing cytochrome P450 enzymes, and modulate the content of glutathione[9].

Literature survey reveals that no any pharmacological studies were conducted to this date to evaluate the hepatoprotective potential of *A.*

**Author for Correspondence: mishra_prati@yahoo.co.in*

Paniculata against cyclophosphamide induced hepatotoxicity. Accordingly, the present investigation designed to assess the healing prospective of ethanolic extract of the *A. Paniculata* (EAPE) on cyclophosphamide induced liver injuries. The study comprises the estimation of the liver function parameters such as serum enzymes- SGPT, SGOT, ALP and total bilirubin content. Antioxidant potential of the EAPE against oxidative stress developed by cyclophosphamide were evaluated with estimation of level MDA, SOD, reduced glutathione and catalase activity in liver tissues homogenates. Liver sections of were examined to assess the healing prospective of the EAPE against the histopathological transformation cause by cyclophosphamide.

METHODOLOGY

Chemicals

Cyclophosphamide Sigma-Aldrich) and silymarin (Sigma- Aldrich) were used in present study. 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 the Agra, Uttar Pradesh. The specimen of *A. Paniculata* identified and authenticated (RARI-JHS/1782-28680) by Regional Research Institute, Jhansi. The whole plant was collected and dried under shade at room temperature and pulverized by mechanical grinder to coarse powder.

Preparation of Plant Extract

Cold maceration method was preferred for the extraction, as to avoid the deterioration of the phytoconstituents. The coarse powder (500gm) of whole plant of *A. Paniculata* successively macerated with petroleum ether (5 lit.) and ethanol (5 lit.). The ethanolic macerated mixture was filtered and filtrate was subjected to dryness under vaccum at 40ºC. The percentage yield were calculated and stored at 4ºC for bioactivity and quantitative analysis.

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 the total phenolic and total flavonoids contents.

Pharmacological Studies

Animals

The present studies were carried out on Wistar rats of weighing 210±10g of either sex and procured from 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 with standard rat pellet diet and water ad libitum. Animal based experimental studies were conducted as per the ethical guidelines of Institutional Animal ethics Committee (Reg. No. 1824/PO/RC/S/15/CPCSEA).

Acute Toxicity Study of HTCE

Acute oral toxicity study of extract of TC was performed as per the OECD-423 guidelines. The extract was administered orally for four dose levels - 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg and observed for the toxic symptoms, body weight changes and lethality. Results are summarized in table no. 1 (LoubnaKharchoufa et.al, 2020).

Hepatoprotective Studies of HTCE Against Cyclophosphamide Induced Toxicity

The hepatoprotective activity of the hydroalcoholic A. paniculata extract evaluated against the cyclophosphamide induced toxicity. Wister strain of albino rats weighing 200gm were selected and divided into 6 groups (n=6 animals). No drug control animal group were considered since hepatoprotective potential of A. **Paniculata Previously Reported.**

Group I: Control treated with normal saline.

Group II: Only 200mg/kg cyclophosphamide introduced intraperitoneally on 1st day.

Group III: 200mg/kg Cyclophosphamide single dose introduced intraperitoneally on 1st day + 250mg/kg hydroalcoholic extract of A. paniculata orally continued for 14 days.

Group IV:200mg/kg Cyclophosphamide single dose introduced intraperitoneally on 1st day + 500mg/kg hydroalcoholic extract of A. paniculata orally continued for 14 days.

Group V: 200mg/kg Cyclophosphamide single dose introduced intraperitoneally on 1st day + 1000mg/kg hydroalcoholic extract of A. paniculata orally continued for 14 days.

Group VI: 200mg/kg Cyclophosphamide single dose introduced intraperitoneally on 1st day + 100mg/kg silymarin orally continued for 14 days.

Serum Preparation for Estimation of the Liver Functional Parameters

After 24 hours of final administration of 14th day of study, the blood was taken from retro-orbital sinus of the experimental animals and placed in Eppendorf Micro-centrifuge tubes and immediately in cooling Micro Centrifuge apparatus at 7000 rpm at 4ºC for 15 minutes to obtain clear serum. The resultant serum was **Transferred in Fresh Sterilized Eppendorf Micro-Centrifuge Tubes and Estimated for the Serum Biochemical**markers- SGPT, SGOT, ALP and total bilirubin content.

Oxidative Stress Studies of HTCE

In the present study the oxidative stress generated by the cyclophosphamide and its numerous metabolites were assessed after 24 hours of final administration of 14th day of study. The animals were sacrificed and their liver tissue were 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 antioxidants assays for Super oxide dismutase (SOD), Catalase activity, Lipid peroxidase, and Glutathione reductase (GSH).

Estimation of SOD

The oxidative stress marker superoxide dismutase estimation based on principle of generation of the superoxide radicals by NADH - phenazinemethosulfate (PMS) system which cause the reduction of tetrazolium salts nitro bluetetrazolium (NBT) into blue formazan which further measured spectrophotometrically at 560nm, against blank. The SOD in the samples competes for the generated superoxide radical, thereby inhibiting the reaction of tetrazoliumreduction[11].SOD activity was determined from the ability of the tissue homogenate to scavenge the superoxide anion generated on cyclophosphamide induced toxicities, as per the method designed by Kakkar et al., 1984. One unit SOD enzyme activity is represented as enzyme concentration needed to inhibit the optical density at 560nm of chromogen production by 50% in one min, and expressed as specific activity in milliunits/mg protein [12].Estimation of lipid peroxidation

The estimation of the lipid peroxidation was conducted to assess the ROS-mediated damage of the cell membranes of the hepatocytes. This has been attributed that under oxidative stress peroxidation of the polyunsaturated fatty acids produces the malondialdehyde (MDA) as end products [11]. Nur Alam et al., 2013). The level of the generated MDA was measured on reaction with thiobarbituric acid (TBARS) in acidic medium at 100^0C as to develop a pink-red colored product which was extracted with butanol: pyridine (15:1) and its absorbance was measured at 520-535nm spectrophotometrically[13].

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 $K_2Cr_2O_7$/acetic acid reagent *(Beers and Sizer, 1952).* The enzymatic activity of the catalase was measured colorimetircally at 610nm as the disappearance of the hydrogen peroxide. Each unit was signifying as the amount that degrades 1 µmol of hydrogen peroxide per minute [11,14].

Estimation of Reduced GSH

Glutathione is a low molecular weight thiol participates in the metabolic protective functions of reduction of the hydroperoxide, detoxification of the xenobiotics and scavenging of the generated free radicals. Ellman developed a method to estimate the level of GSH (G L Ellman, 1959), based on the ability of the 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman reagent) to react with compounds containing sulfhydryl groups, to produce 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 blank. Absorbance values were compared with a standard curve generated from known GSH[11].

Histopathological Investigation

The liver tissues were immersed in 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 the pathological findings.

Statistical Analysis

Results are provided as Mean ± SD (n=6). Results were analyzed statistically using one-way analysis of variance (ANOVA) followed by Bonferroni t-test. $P < 0.05$ was considered as level of significance while comparison between groups.

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%. Qualitative test for phytochemicals confirmed the presence of alkaloids, saponins, tannins, glycosides, terpenoids, steroids, flavonoids and phenolic compounds. Quantitative estimation for total phenolic content - 86.50mg/gm equivalent to gallic acid and total flavonoid content-68.50mg/gm equivalent to rutin were reported.

Acute Toxicity of Assessment of HTCE

Acute toxicity studies conducted to assess the safety aspect of EAPE. Neither any mortality nor any symptoms of toxicity up to the dose of 2000mg/kg were reported during 72hrs observation of animals of all groups. This complies with the previous results of *A. paniculata*[15], which signifies safety of EAPE up to the dose of 2000mg/kg.

Table 1: Acute toxicity studies of EAPE

Group	Dose (mg/kg)	Rat No.	Day of Death	Body Weight (gm) 0 Day	7 Day	14 Day	No. Death/Tested
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 Hepatoprotective Potential of HTCE

Hepatotoxicity is a major side effect of cyclophosphamide. Cyclophosphamide undergoes extensive hepatic metabolism by Cyp-450 mixed function oxidase system to produce active metabolites including 4-hydroxycyclophosphamide, phosphoramide mustard and acrolein[16]. Cyclophosphamide and its metabolites distorted hepatic cell membrane integrity by initiating oxidative degradation of lipids of cell membranes, resulting hepatic injuries with leaching out of the hepatocellular contents and enzymes such as SGOT, SGPT, ALP as well as bilirubin into systemic circulation[16].

The results of serum biochemical parameters for group II animals (treated with cyclophosphamide) reported a remarkable elevation in SGOT, SGPT and ALP. This indicates an intense hepatic injuries produced by cyclophosphamide. Elevation of AST and ALP 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 enzymes level SGOT, SGPT and ALP (table 2) in a dose dependent manner, this signifies that *A. paniculata* have hepatic wound healing potential. The hepatic healing effect of *A. paniculata* at dose of 1000mg/kg fairly near to the effect produces by the pure standard drug silymarin. Supplementation with EAPE at dose of 250mg/kg decreases the elevated level of enzymes upto 50%, while restored to the normal at dose of 1000mg/kg. This signifies that the 250mg/kg could be considered as a minimum dose for significant hepatoprotective effect of *A. Paniculata.*

Increase in Bilirubin content indicates the incidence of hepatic necrosis and its accumulation characterized as hepatocytes functional insufficiency, biliary obstruction or increase in the haemolysis. Administration of Cyclophosphamide also reported for severe sinusoidal obstruction syndrome, characterised with hepatic necrosis, obstruction of the hepatic venous flow and jaundice[17, 18]. Raised in the serum level of the bilirubin indicate the prevalence of the jaundice. Cyclophosphamide increase the total bilirubin form 0.3 ± 0.03 in control group to 2.11 ± 0.19 (mg/100ml of serum), this indicates that cyclophosphamide alter the metabolism of the bilirubin. Treatment with *EAPE* re-establishes the serum bilirubin level in dose dependent manner and at dose of 1000mg/kg serum bilirubin level restored fairly near to normal.

Table 2: Evaluation of Hepatoprotective potential of the EAPE.CYP, Cyclophosphamide (200mg/kg); SIL, Silymarin (100mg/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. Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, ns, p< 0.01, non-significant activity and *P<0.050, **P<0.001 significant activity when compared to the control.

	Treatment		Liver Function Parameters						
Groups	Day "0" / dosing mg/kg BWT	Day "1-21" / dosing mg/kg BWT	Body weight (gm)	Absolute liver wt. (gm)	Rel. liver wt. (gm/100 gm)	SGOT (IU/dL)	SGPT (IU/dL)	ALP (IU/dL)	Bilirubin (mg/dL)
I	Vehicle (i.p)	Only animal feed. (Oral)	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	200mg CPP (i.p)	Only animal feed (Oral)	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	200mg CPP (i.p)	100mg EAPE (Oral)	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	200mg CPP (i.p)	250mg EAPE(Oral)	201.42±3.45 **	5.17±1.17 **	2.56±0.53 **	57.75±8.42 **	42.14±4.93 **	128.37±13.58 **	0.71±0.19 **
V	200mg CPP (i.p)	500mg EAPE(Oral)	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	200mg PP (i.p)	1000mg EAPE(Oral)	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 **

Evaluation of Oxidative Stress of HTCE

Oxidative stress is a state characterized with the elevation of the intracellular ROS (reactive oxygen species). Reactive oxygen species (ROS) are the common by- products as chemical molecules containing oxygen which 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 into hepatic cellular health and contribute into liver cirrhosis by inducing the destruction of the DNA, proteins and lipids peroxidation. Liver has a classical antioxidant enzyme defense system of superoxide dismutase (SOD), scavenges superoxide anions and reduce the toxic and deleterious effect of the free radical; glutathione reductase (GS), pivotal enzyme for maintaining and recover the reduce levels of glutathione in cytoplasm; lipid peroxidase, and catalase, decomposes H_2O_2 and protects the tissue

form highly reactive hydroxyl radicals. Imbalance between the ROS production and antioxidant defense mechanism of liver may leads to hepatic injuries. Oxidative stress biomarkers are therefore important tools to assess the hepatic cellular health.

This has been established that extensive hepatic metabolism of the cyclophosphamide into acroline leads hepatocellular toxicity. Cyclophosphamide toxicity decreases the level of the SOD, GSH and CAT below to the normal; this indicates the over accumulation of free radicals[**16]**.Results of oxidative stress studies, shown in table no. 3, reveals that cyclophosphamide causes excess of lipid peroxidation, with 3-fold amplification in level of malondialdehyde (MDA), along with fall in the level of the SOD, GSH; as well as declination in the CAT activity when compared with control group[**19]**. Treatment with EAPE extract restored the raised level of SOD, GSH and CAT activity in dose dependent manner and at dose of 1000mg/kg the level reached near to normal. This signifies the ability of *A. paniculata* against cyclophosphamide toxicity to establish a balance between the hepatic antioxidant enzyme defense system and production of the ROS

.

Table 3: Oxidative Stress result. EAPE, ethanolic extract of *A. Paniculata*; CPP, cyclophosphamide; SIL, Silamyrin; LPO, Lipid peroxidise (nmol MDA/mg tissue); SOD, superoxide dismutase (units/min/mg protein); GSH, Glutathione reductase (1 µM of NADPH/min); CAT, Catalase (µm H_2O_2 consumed/min/mg protein). Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ^{NS}P>0.001 compared to the control.

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	Vehicle + CPP	50.78±2.05	10.57±3.53	0.41±0.02	12.42±0.81
III	CPP +Sample EAPE 250	36.47±1.98**	23.05±2.28**	1.16±0.01**	16.98±1.85 ns
IV	CPP +Sample EAPE 500	22.22±1.39**	29.42±5.13**	1.21±0.01**	22.43±2.86**
V	CPP +Sample EAPE 1000	17.11±1.02**	36.2±2.85**	1.42±0.01**	26.26±4.45**
VI	CPP+ SIL 100 mg/kg	15.69±1.82**	50.16±5.41**	2.09±0.04**	36.65±2.86**

Histopathology Examination of HTCE

Hepatic histopathology examined to evaluate the hepatoprotective effect of *EAPE* against cyclophosphamide induced toxicity. Liver section of animals of control (Group –I) showed a normal hepatic architecture with radially arranged hepatic cords around the central vein. Animals received single dose of 200mg/kg of Cyclophosphamide (Group II) developed a severe hepatic toxicity evidenced with severe necrosis, hepatic congestion, and inflammation. There were also indications of intense granulocytic infiltration. Liver section of Animals treated with 250mg/kg of EAPE (Group III) reveals a some extent of healing effect with initiation of regeneration and restoration of the the normal heaptocytes parenchyma. Animals kept on treatment with 500mg/kg of EAPA (Group-IV) reveals a remarkable regeneration and restoration of hepatic architecture. Liver section of animals treated with 1000mg/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 (100mg/kg) showed normal heaptic architecture.

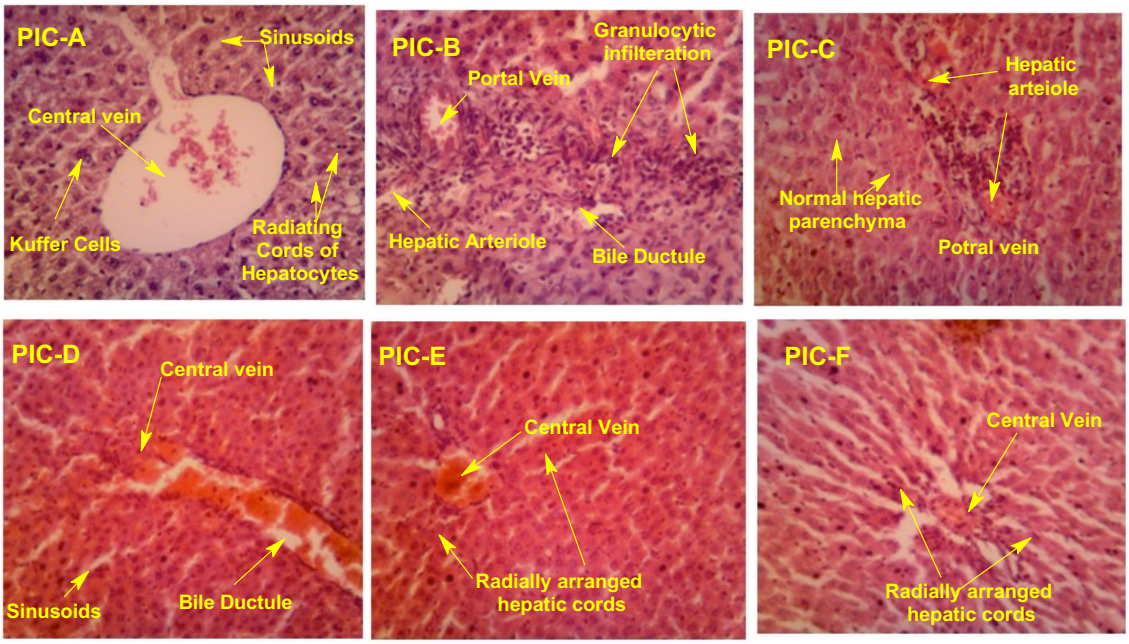

Figure 1: Histological Investigation of liver sections. PIC-A, Group I (Control-Dist. water); PIC-B, Group II (Cyclophosphamide-200mg/kg); PIC-C, (EAPA-250mg/kg); PIC-D (EAPA-500mg/kg); PIC-E,(EAPA-1000mg/kg); PIC-F (Silymain-100mg/kg).

CONCLUSIONS

The results of present study reveal that CPA exposure elevates the level of serum liver functional enzymes and develop the oxidative stress along with the distortion of hepatic architecture. Supplementation with *A. paniculata* restores the level of serum liver functional enzymes by preventing LPO and reverses the oxidative stress by enhancing the antioxidant defence system. Based on the results of present study *A. paniculata* could be consider as a supplement during CPA chemotherapy as to minimise hepatic toxicities.

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

Acknowledgement

The authors would like to express their cordial thanks Regional Research Institute, Jhansi for the authentication of the plant.

Conflict of Interest

The author declares that there is no any conflict of interest.

Author (s) Contribution

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

REFERENCES

1. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Pharmaceuticals. Lyon (FR): International Agency for Research on Cancer; 2012. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 100A.)CYCLOPHOSPHAMIDE.
2. Starzl, T E et al. “Cyclophosphamide and whole organ transplantation in human beings.” Surgery, gynecology & obstetrics vol. 133,6 (1971): 981-91 https://www.researchgate.net/publication/18227280.
3. Boddy AV, Yule SM, "Metabolism and pharmacokinetics of oxazaphosphorines".Clinical Pharmacokinetics, 38 (4) (2000) 291–304).
4. Whitney A. High,19 - Cytotoxic Agents, Editor(s): Stephen E. Wolverton, Comprehensive Dermatologic Drug Therapy (Fourth Edition), Elsevier, 2021, Pages 209-221.e5.
5. King PD, Perry MC, Hepatotoxicity of chemotherapy, Oncologist, 6 (2001) 162-76.DOI: 10.1634/theoncologist.6-2-162

6. Adams JD Jr, Klaidman LK, Acrolein-induced oxygen radical formation, Free RadicBiol Med, 15 (2) (1993) 187-93.
7. Md. Sanower Hossain, Zannat Urbi, AbubakarSule, K. M. Hafizur Rahman, "Andrographispaniculata (Burm. f.) Wall. exNees: A Review of Ethnobotany, Phytochemistry, and Pharmacology", The Scientific World Journal, vol. 2014, Article ID 274905, 28 pages, 2014.
8. Liu YT, Chen HW, Lii CK, Jhuang JH, Huang CS, Li ML, Yao HT. A Diterpenoid, 14-Deoxy-11, 12-Didehydroandrographolide, in Andrographispaniculata Reduces Steatohepatitis and Liver Injury in Mice Fed a High-Fat and High-Cholesterol Diet. Nutrients. 2020 Feb 18;12(2):523.
9. W. Chao and B. Lin, "Hepatoprotective Diterpenoids Isolated from Andrographis paniculata," Chinese Medicine, 3 (3) (2012) 136-143.
10. Harbone JB, Phytochemical methods - A guide to modern technique of plant analysis, 2nd edn, Chapman and Hall, Newyork, 1984, p. 1-85. ISBN-13: 978-0-412-23050-9 e-ISBN-13: 978-94-009-5921-7
11. Md. Nur Alam, Nusrat Jahan Bristi, Md. Rafiquzzaman, Review on in vivo and in vitro methods evaluation of antioxidant activity, Saudi Pharmaceutical Journal, 21(2) (2013) 143-152,
12. Poonam Kakkar, Ballabh Das, PN Viswanathan, A modified Spectrophotometric Assay of Superoxide Dismutase, Indian Journal of Biochemistry & Biophysics, 21 (1984) 130-132.
13. Ohkawa H, Ohishi N, Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal Biochem, 95 (2) (1979) 351-8.
14. Sinha AK. Colorimetric assay of catalase.Anal Biochem, 47 (2) (1972) 389-94.
15. Worasuttayangkurn L, Nakareangrit W, Kwangjai J, Sritangos P, Pholphana N, Watcharasit P, Rangkadilok N, Thiantanawat A, Satayavivad J, Acute oral toxicity evaluation of Andrographis paniculata-standardized first true leafethanolic extract, Toxicol Rep, 6 (6) (2019) 426-430.
16. Oyagbemi AA, Omobowale OT, Asenuga ER, Akinleye AS, Ogunsanwo RO, Saba AB, Cyclophosphamide-induced Hepatotoxicity in Wistar Rats: The Modulatory Role of Gallic Acid as a Hepatoprotective and Chemopreventive Phytochemical, Int J Prev Med, 1 (7) (2016) 51.
17. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012.
18. Eva D. Papadimitraki, Dimitrios T. Boumpas, Chapter 46 - Cytotoxic Drug Treatment, Editor(s): George C. Tsokos, Caroline Gordon, Josef S. Smolen, Systemic Lupus Erythematosus, Mosby, 2007, p.498-510.
19. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:360438.