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Protective Effect Of *Neptunia triquetra* Extract On Hepatotoxicity Induced By A Chemotherapeutic Drug Cyclophosphamide In Rats

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Abstract

Cyclophosphamide is the chemotherapeutic drug used to treat several types of cancers, it has many side effects among them hepatotoxicity is one of the important side effect taken in to consideration because, it is the condition in which impairment of normal liver function occurs that might leads to severe hepatic damage, if not diagnosed properly. Currently there is no therapy for CPA induced hepatotoxicity other than supportive treatment. Generally the imbalance between oxidant and antioxidant in the body is one of the important source of toxicities, developed by certain drugs and its metabolites. The medicinal plants, its extracts with antioxidant compounds are safe and have capacity to protect the body from various toxicities. So this study was aimed to screen the protective effect of *Neptunia triquetra* extract on cyclophosphamide induced hepatotoxicity. Rats were intoxicated with a single dose of CPA (200 mg/kg, by i.p) on 15th day of treatment to induce hepatotoxicity and it was confirmed by the altered levels of biochemical, hematological parameters along with histopathological changes in liver tissue. Treatment of rats with methanolic extract of *Neptunia triquetra* at 100, 200 and 400 mg/kg, p.o for 15 days significantly protected the CPA induced hepatotoxicity as evidenced by restoration of all altered levels of biochemical, hematological parameters along with improved histopathological findings. From the results it was concluded that NTME protected the liver from CPA induced hepatotoxicity and the test dose of 400 mg/kg exhibited significantly more protective effect compared to the doses of 100 and 200 mg/kg.

Keywords: *Neptunia triquetra*, Protective effect, Hepatotoxicity, Cyclophosphamide, Hepatic marker enzymes.

Introduction

Chemotherapy is the effective treatment to cures the cancer by destroying, preventing the growth of the cancer cells. From the last few decades many chemotherapeutic drugs have been developing for its effectiveness. The drugs used for chemotherapy are powerful, along with cancer cells they can act on healthy cells includes, cells in the blood, mouth, digestive system, hair follicles etc. then causes damage leads to side effects of chemotherapy. Different drugs of chemotherapy causes different side effects but certain drugs have specific side effects. Some drugs can induce significant liver injury. In this, direct chemotherapy induced hepatotoxicity is the important side effect to focus. Many chemotherapeutic drugs require adequate liver function because these drugs acts directly on the liver for its metabolism, activation and clearance. In the treatment of cancer many side effects will observe and the main difficult problem is determining the cause of abnormal liver function. Hepatotoxicity from chemotherapy occurs frequently and it is unpredictable, which leads to structural damages of liver sinusoids, bile ducts, hepatocytic damage, vascular occlusion, accumulation of toxic metabolites, inflammatory cell infiltration and long term complications such as complete liver failure or cirrhosis was observed if unnoticed, and also pre-existing liver problems can further increases the toxicity of liver. So careful monitoring is required such as dose modification, administration of supportive drugs, drug discontinuation and continuous examination of liver function during administration of chemotherapeutic drugs(Alla & Christopher, 2014; Giuliano & Silke, 2010;Ankush, et al.,2014; Delco, et al.,2005).

Cyclophosphamide(CPA) is a chemotherapeutic drug belongs to the class of alkylating agent, derivative of nitrogen mustard. It is an antineoplastic and immunosuppressive agent used to treat several types of cancers including lymphoma, leukemia, multiple myeloma, sarcoma, neuroblastoma, breast and ovarian cancers and also treats nephrotic syndrome(Bethesda, 2012) CPA can be given as oral, intravenous routes. The common side effects of CPA include alopecia, nausea, vomiting, diarrhea, cystitis, oral ulcers, bone marrow suppression. Severe side effects are idiosyncratic, not common, rare include severe neutropenia, sepsis, cardiotoxicity, hepatotoxicity, hemorrhagic cystitis, embryo- fetal toxicity and secondary malignancies(Ogino & Tadi, 2021). CPA is a prodrug, it needs metabolic activation for therapeutic action. The metabolism and activation takes place in the liver. The drug is undergo metabolism with hepatic enzyme cytochrome P450 isoforms and converts to several intermediate metabolites. 4-hydroxy cyclophosphamide is one of the main active metabolite which is further metbolized in to aldophosphamide. Aldophosphamide further converts in to active alkylating metabolite phosphoramide mustard and acrolein (Deleve,2013). The anticancer activity of CPA is due to

Phosphoramide metabolite it shows its effect through the alkylation process and forms cross linkages in the DNA strands leads to inhibition of protein synthesis this results in to programmed death of the cell, while acrolein is chemically α, β- unsaturated aldehyde, highly reactive toxic metabolite it produces its toxic effect to all the cells which are exposed to acrolein and also it was identified as the initiator of lipid peroxidation, inducer of oxidative stress this may change the biochemical and physiological factors, finally leads to toxicity. All these parameters limit the use of CPA in clinical purpose.

In CPA treatment mild elevation in the levels of serum aminotransferase commonly observed. In high dose, the serum enzymatic elevations are common but clinically apparent liver injury with standard dose is not common, so several reports have been publishing that, in CPA treatment acute liver injury with jaundice was observed (Bethesda, 2012). Generally in chemotherapy of cancer, high doses of CPA recommended in the combination of total body irradiation in cell transplantation therapy this can leads to sinusoidal obstruction syndrome this may be the direct toxic effective action of CPA on sinusoidal cells of liver which leads to cell necrosis and release in to the sinusoids, obstruction, obliteration of hepatic veins this can be severe leading to acute liver failure and death (Subramaniam, et al., 2013).

The severity of liver toxicity with CPA ranges from mild elevations of liver enzymes to acute liver injury and fatal hepatic necrosis due to sinusoidal obstruction syndrome(Linda, et al., 1993) There is currently no specific therapy for CPA produced liver injury other than supportive treatment and avoidance of further damage by early diagnosis. This diagnosis is based on the symptoms of severity like tenderness, weight gain, ascites, liver enlargement, hepatic dysfunction and jaundice (Bethesda, 2012).

The usage of medicinal plants has been increasing day by day for their bioactive compounds. These compounds acts effectively, protects safely from different toxicities (Abayomi, et al., 2013). The presence of natural antioxidant compounds mainly polyphenols, flavonoids, anthocyanins in medicinal plants are the important constituents which prevents the oxidative damage to the cells. The imbalance between oxidants and antioxidants in the body is the main cause of the toxicities which is developed by the metabolites of certain drugs. So the plant extracts or herbal drugs with antioxidants are safe, inexpensive and available from natural resources with no side effects and have the capacity to protect the body from various toxicities (Ammar, et al.,2017).

Neptunia triquetra (Vahl) Benth. (Family: Leguminosae) commonly known as Yellow sensitive plant usually found in India among the grasses in cultivated lands. It is a small perennial, low prostrate herb with 1-2 pairs pinnae, 2cm long, 8-12 pairs leaflets, oblong rounded at apex with slender ascending

compressed stems, yellow flowers, oblong flat beaked pods with 4-6 seeded. The entire plant is edible and used medicinally for various purposes. Flowers were used to cure eye infections, fresh leaf juice is used as refrigerant, young stems are acts as a stimulant, astringent (Maheshwari, 2000) and its juice is used in earache. The whole plant is good tonic and used particularly in the treatment of jaundice, intestinal diseases such as diarrhoea and dysentery(Singh, 2007). Based on available research reports of the plant, the in-vitro antioxidant [Ahmed, et al., 2019) and antibacterial (Ahmad, et al., 2019) activities were only screened from the extract of leaves. This reveals the necessary of exploring this medicinal plant scientifically.

Medicinal plants and its extracts are plays a vital role in the treatment of diseases and very much helpful, suitable compared to existing preventive treatments. The protective activity of *Neptunia triquetra* extract against CPA induced hepatotoxicty have not been reported earlier. So the present research was aimed to screen the protective effect of *Neptunia triquetra* extract on hepatotoxicity induced by a chemotherapeutic drug cyclophosphamide in rats.

Materials and Methods

Drugs and chemicals

CPA was obtained in powder form, from Shilpa Medicare Ltd., Raichur, Karnataka, India. Silymarin was purchased from Sigma-Aldrich, China. Biochemical kits for estimation of AST, ALT, ALP, ALB, TP, TB, DB, LDH were purchased from Merck specialities Pvt. Ltd., Mumbai, India. TCA and TBA were purchased from Himedia, Mumbai, India and all the used remaining chemicals and reagents were analytical grade.

Collection and extraction of plant material

The whole plant of *Neptunia triquetra* was collected from the surroundings in Chittor district, Andhra Pradesh, India. The plant material was authenticated by Dr.K.Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India. A voucher specimen of the plant having number KU/UCPSC/54 was kept in the herbarium of Department of Pharmacognosy and Phytochemistry in University college of Pharmaceutical sciences, Kakatiya University, Warangal. One kilogram of fresh plant material was taken and washed under running tap water, shade dried and coarsely powdered. This powdered plant material was extracted with methanol by maceration technique for seven days and filtered. The filtrate was concentrated to dryness by using rotary evaporator and percentage yield was calculated (9.5%) then stored in desiccator. The obtained methanolic extract was tested for various

phytoconstituents like alkaloids, glycosides, flavonoids, tannins, terpenoids, saponins etc. by using different chemical tests.

Estimation of total phenolic content

The total phenolic content in methanolic extract of *Neptunia triquetra* (NTME) was estimated by using Folin-Ciocalteu colorimetric method using gallic acid as a standard and the amount of total phenolics was expressed in terms of gallic acid equivalent (GAE) (Samatha, et al., 2012).

Estimation of total flavonoid content

The total flavonoid content in NTME was estimated by using Aluminium chloride colorimetric method using rutin as a standard and the amount of total flavonoids was expressed in terms of rutin equivalent (RE) (Samatha, et al., 2012).

Experimental animals

Both Male and Female Wistar rats weighing 150-200 grams were purchased from Vyas Labs, Hyderabad, India. Then kept in polypropylene cages and housed for acclimatization at 22 ± 3^0 C with a 12hour light/dark cycle for one week prior to the experiment with permission from institutional animal ethical committe. (IAEC/12/UCPSC/KU/2020) Rats were fed with standard pelleted diet, drinking tap water *ad libitum.*

Acute toxicity study

Acute toxicity study was performed on the methanolic extract of plant according to the OECD - 423(2001) guidelines. Female Wistar rats were used in this study. The animals were fasted overnight with only water accessible before administration of test dose. All the Animals were observed individually after dosing, during the first 24hours and then daily for 14 days to observe the mortality and signs of toxicity.

Experimental design

Wistar male rats were randomly divided in to six groups with six animals in each group(n=36). Treatment of each group as follows: group I- normal control, animals of this group were injected with normal saline by intraperitoneal route, once daily for 15 days. Group II- was toxic control and they were left for 14 days without any treatment then intoxicated with a single dose of cyclophosphamide to induce

hepatotoxicity (200 mg/kg, by i.p) on 15th day of treatment, group III- Standard group, in which animals were treated with silymarin (100 mg/kg, p.o) once daily for 15 days then received a single dose of CPA on 15th day. Group IV, V and VI- Treated (Tested) groups1,2 and 3 animals of which received methanolic extract of *N.triquetra* (100, 200 and 400 mg/kg p.o) once daily for 15 days then received a single dose of CPA on 15th day. After administration of last dose, animals were allowed to fast overnight. On the next day whole blood was withdrawn from the rats by sino-orbital puncture with the overdose of diethyl ether then the animals were sacrificed and liver was separated immediately and rinsed in cold saline, blotted, dried, weighed and used for preparation of liver homogenate, histopathological findings (Ahmed, 2018; Dina, et al., 2017).

Analysis of Biochemical Parameters

Analysis of Serum Biochemical Parameters

The collected blood was allowed to coagulate at room temperature then centrifuged at 3000 rpm for 10min to separate the serum. The obtained serum was used for estimating the biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatise (ALP), total bilirubin (TB), direct bilirubin (DB), total protein (TP), albumin (ALB), lactate dehydrogenase (LDH) and lipid profile parameters (Peter, 2003) including HDL-cholesterol, LDL-cholesterol, Total cholesterol, triglycerides content by using commercially available standard assay kits with autoanalyzer.

Analysis of Hepatic Tissue Biochemical Parameters

A portion of collected liver tissue (10%) was homogenised with basic phosphate buffer (pH 7.4) by using tissue homogenizer and homogenate was centrifuged at 3000rpm for 15 min at 4^{o} C. The obtained homogenate was used to estimate the lipid peroxidation (LPO)(Hiroshi, et al., 1979), superoxide dismutase(SOD)(Poonam, et al., 1983), catalase(CAT) (Asru, 1972), glutathione(GSH) (George, 1959) glutathione dependent enzymes such as glutathione peroxidase(GPx) (Rotruck, et al., 1973) glutathione S-transferase(GST) (Habig, et al., 1974) and glutathione reductase(GR) (Staal, et al., 1969) levels by using standard procedures.

Analysis of Hematological parameters

Red blood cells(RBCs), White blood cells(WBCs) and Hemoglobin(Hb) contents were estimated by using blood sample which was collected in test tube containing disodium salt of EDTA.

Analysis of Liver Histopathology

The remaining portion of collected liver tissue was fixed in 10% buffered neutral formalin solution, embedded in paraffin, cut in to sections of 3-5µm and stained with hematoxylin-eosin. Finally microscopic observation was done by using Digital Motic Microscope under 100X magnification.

Statistical analysis

The results of data obtained were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's method using graph pad prism 9.0 and all the results were expressed as mean±Standard deviation (SD). The value of $p<0.05$ was considered as statistically significant.

Results

Phytochemical analysis

Preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, steroids, flavonoids, terpenoids and phenolic compounds in methanolic extract. The total phenolic content in extract was found to be 21.12±1.14 mg of GAE per gram of dry extract and the total flavonoid content in extract was found to be 55.75±2.45 mg of RE per gram of dry extract.

Acute toxicity study

Rats administered with NTME did not show any toxicity symptoms during the first 24hours and no mortality occurred until the period of 14 days with a dose level of up to 2000 mg/kg body weight.

Effect of NTME on body weight and liver weight

The effect of NTME on body weight and liver weight in CPA induced hepatotoxicity in rats was shown in table 1. The final body weight of rats intoxicated with CPA significantly decreased compared with normal control. Groups treated with NTME at 100, 200 and 400 mg/kg and standard drug silymarin at 100 mg/kg significantly increased the body weight compared with the toxic group. The Liver weight, relative liver weight of rats induced with CPA increased significantly when compared with normal rats. Significantly decreased liver weight and relative liver weight was observed in the groups of rats treated with test doses, standard dose compared to toxic group of rats.

Effect of NTME on serum biochemical parameters

The effect of NTME on biochemical parameters of serum was shown in table 2. Induced hepatotoxicity was observed in CPA treated group with significantly increased levels of AST, ALT, ALP, LDH, TB, DB and decreased TP, ALB levels were observed compared to normal group. In standard, extracts treated test groups the AST, ALT, ALP, LDH, TB, DB levels were decreased and TP, ALB levels were increased significantly when compared to toxicity induced group, it indicates the standard and test groups were protected from hepatotoxicity caused by CPA. From the three test doses, NTME at 400 mg/kg was better protected and it was well comparable to that of standard drug silymarin at 100 mg/kg.

Effect of NTME on serum lipid profile

The effect of NTME on lipid profile of serum in CPA induced hepatotoxicity was shown in table 3. Rats treated with only CPA showed significant elevated levels of cholesterol, triglyceride, LDL-cholesterol and depleted levels of HDL-cholesterol when compared to normal control. Rats treated with extracts, standard along with CPA showed significantly decreased levels of cholesterol, triglyceride, LDL-cholesterol and increased levels of HDL-cholesterol as compared to toxic control indicates that rats treated with standard and test doses showed better protection from toxicity without any altered lipid profile parameters.

Effect of NTME on hepatic oxidant and antioxidant parameters

The effect of NTME on oxidative stress parameters of liver tissue protein in CPA induced hepatotoxicity was shown in table 4. The hepatic CAT, GSH, SOD, GPx, GST and GR levels were significantly decreased and hepatic LPO levels in tissue homogenate were increased in toxic group II compared to normal group I. When treated with standard dose in group III, test doses in group IV, V, VI along with CPA, the activities of CAT, GSH, SOD, GPx, GSTand GR were significantly enhanced and the activity of LPO was significantly reduced as compared to toxic alone in group II.

Effect of NTME on hematological parameters

The effect of NTME on hematological parameters in CPA induced hepatotoxicity was shown in table 5. The count of RBCs and WBCs were decreased significantly along with the Hb content in the toxic group, induced alone with CPA compared with normal group. In group IV, V and VI the decreased levels of RBCs, WBCs and Hb content were increased significantly compared with toxic group.

Table 1. Effect of NTME on body weight and liver weight in CPA induced hepatotoxicity in rats.

Groups	Initial body weight(g)	Final body weight(g)	Liver weight(g)	Relative liver weight(%)
I –Normal	156.5±6.56	182.3±7.85	5.45±0.23	2.98±0.11
II –Toxic	173.8±4.75	138.5±7.41[a]	8.55±0.15[a]	6.17±0.06[a]
III-Standard	163.6±5.54	187.8±4.81[b]	5.77±0.19[b]	3.07±0.12[b]
IV-Test1	168.4±4.84	173.5±5.61[c]	7.95±0.21[c]	4.58±0.11[c]
V -Test2	178.7±7.12	192.5±7.22[b]	7.16 ±0.12[b]	3.71±0.10[b]
VI -Test3	180.7±5.33	198.8 ± 8.21[b]	6.10±0.09[b]	3.06±0.15[b]

Values are expressed as mean ± SD, n=6. [a]P<0.001 values-compared to normal control. [b]P<0.001, [c]P<0.01, [d]P<0.05 values-compared to toxic control.

Table 2. Effect of NTME on biochemical parameters of serum in CPA induced hepatotoxicity

Groups	AST(U/L)	ALT(U/L)	ALP(U/L)	LDH(U/L)	TB(mg/dl)	DB(mg/dl)	TP(g/dl)	ALB(g/dl)
I -Normal	46.56±2.55	50.57±2.73	151.98±6.44	170.14±5.26	0.58±0.13	0.29±0.08	5.95±0.29	3.55±0.44
II -Toxic	84.66±3.97[a]	100.91±4.75[a]	239.17±8.15[a]	397.63±6.51[a]	2.13±0.11[a]	1.99±0.15[a]	1.91±0.12[a]	1.92±0.14[a]
III-Standard	51.13±3.18[b]	53.71±2.48[b]	159.22±4.72[b]	180.23±4.57[b]	0.61±0.13[b]	0.32±0.18[b]	5.81±0.11[b]	3.37±0.45[b]
IV-Test1	70.55±3.18[c]	90.95±5.38[c]	195.57±6.85[c]	310.37±7.91[c]	1.53±0.14[c]	0.91±0.09[c]	2.79±0.91[c]	2.45±0.21[c]
V -Test2	63.78±2.45[b]	80.78±4.15[b]	171.57±5.64[b]	219.41±6.56[b]	0.92±0.08[b]	0.63±0.04[b]	4.25±0.77[b]	2.95±0.31[b]
VI -Test3	55.33±2.15[b]	60.33±4.18[b]	164.72±4.51[b]	195.55±5.54[b]	0.69±0.09[b]	0.39±0.07[b]	5.24±0.54[b]	3.15±0.17[b]

Values are expressed as mean ± SD, n=6. [a]P<0.001 values-compared to normal control. [b]P<0.001, [c]P<0.01, [d]P<0.05 values-compared to toxic control.

Table 3. Effect of NTME on lipid profile of serum in CPA induced hepatotoxicity

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)
I –Normal	55.94±2.15	63.26±1.9	35.85±1.14	22.95±0.64
II –Toxic	101.54±4.82[a]	115.45±4.24[a]	15.43±0.91[a]	50.57±3.55[a]
III-Standard	63.54± 2.92[b]	68.98±3.59[b]	33.13±2.10[b]	25.61±0.38[b]
IV-Test1	85.63±3.27[c]	92.51±3.25[c]	21.88±1.14[c]	41.53±1.14[c]
V -Test2	78.53±2.98[b]	85.82±3.53[b]	25.47±1.19[b]	35.54±2.14[b]
VI -Test3	65.84±2.45[b]	72.97±2.75[b]	30.11±1.85[b]	28.94±1.33[b]

Values are expressed as mean ± SD, n=6. [a]P<0.001 values-compared to normal control. [b]P<0.001, [c]P<0.01, [d]P<0.05 values-compared to toxic control.

Table 4. Effect of NTME on oxidant and antioxidant parameters of hepatic tissue in CPA induced hepatotoxicity

Groups	CAT(U/mg)	SOD(U/mg)	GSH(mM/mg)	LPO(mM/mg)	GPx (U/mg)	GST(mU/mg)	GR(U/mg)
I -Normal	14.95±0.22	12.94±0.11	5.55±0.29	2.14±0.13	12.15±0.51	4.54±0.05	60.24±2.55
II -Toxic	4.45± 0.11[a]	5.55±0.24[a]	1.33±0.22[a]	5.52±0.12[a]	7.54±0.21[a]	2.21± 0.02[a]	31.55±1.04[a]
III-Standard	13.68±0.14[b]	12.13±0.18[b]	5.25±0.31[b]	2.48±0.12[b]	11.42±0.29[b]	4.05±0.01[b]	58.24±2.01[b]
IV-Test1	7.54±0.13[c]	6.96±0.41[c]	3.14±0.19[c]	4.63±0.19[c]	8.42±0.42[c]	2.85±0.04 [c]	38.54±1.05[c]
V -Test2	10.14±0.15[b]	9.18±0.33[b]	4.26±0.13[b]	3.96±0.21[b]	9.22±0.57[b]	3.45±0.12[b]	45.87±2.04[b]
VI -Test3	12.96±0.10[b]	11.75±0.43[b]	5.01±0.21[b]	2.98±0.14[b]	10.54±0.21[b]	3.95±0.21 [b]	54.74±1.08[b]

Values are expressed as mean ± SD, n=6. [a]P<0.001 values-compared to normal control. [b]P<0.001, [c]P<0.01, [d]P<0.05 values-compared to toxic control.

Table 5. Effect of NTME on hematological parameters in CPA induced hepatotoxicity

Groups	RBCs(10^6/µL)	WBCs(10^3/µL)	Hb(g/dL)
I -Normal	6.55±0.41	10.54±0.75	12.55±0.82
II –Toxic	3.45± 0.33[a]	5.25±0.81[a]	8.63±0.44[a]
III-Standard	6.48±0.24[b]	10.43±0.48[b]	11.95±0.51[b]
IV-Test1	4.64±0.23[c]	6.56±0.55[c]	9.24±0.59[c]
V -Test2	5.44±0.31[b]	8.58±0.33[b]	10.66±0.23[b]
VI -Test3	6.26±0.45[b]	10.15±0.53[b]	11.69±0.22[b]

Values are expressed as mean ± SD, n=6. [a]P<0.001 values-compared to normal control. [b]P<0.001, [c]P<0.01, [d]P<0.05 values-compared to toxic control.

Effect of NTME on Histopathology of liver

The effect of NTME on histopathology of liver in CPA induced hepatotoxicity was shown in figure1. In normal rats the hepatocytes are appeared normal centrilobular region with normal sinusoidal spaces and central vein, bile ducts are also appeared normal. The hepatotoxic rats induced with CPA showed abnormal in structures, involves single and multi focal centrilobular necrosis of hepatocytes, dilation of the sinusoidal spaces with hemorrhages, peri biliary fibrosis and infiltration of inflammatory cells were observed and also appeared fatty deposits with dilated central vein. In treated groups according to given doses Test1 shows mild, Test2 shows moderate and Test3 shows good recovery such as normal hepatocytes, mild peri biliary infiltration of inflammatory cells with less fatty changes, improvement in hepatic necrosis, dilation in central vein and sinusoidal dilation. In standard group, the rats showed adequate hepatic architecture without any abnormalities and degenerative changes of cells.

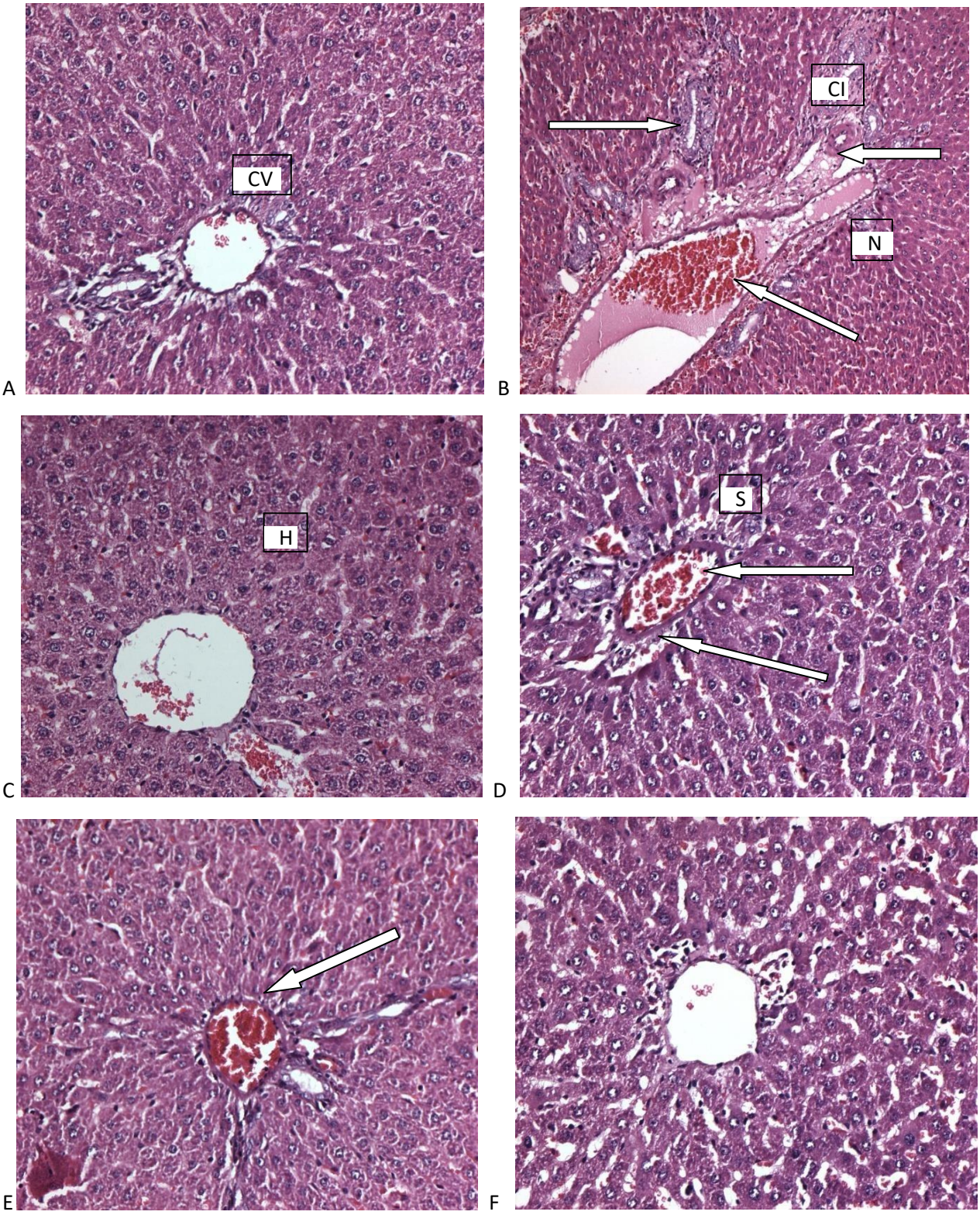

Fig. 1. Effect of NTME on histopathology of liver in CPA induced hepatotoxicity

Histopathological changes of liver tissue observed under 100X magnification by using Digital Motic Microscope with hematoxylin-eosin stain in normal and treated groups of rats. A. Normal group- showing normal central vein(CV) with normal hepatocytes(H). B. Toxic group- showing abnormal structure with centrilobular hepatic necrosis(N), hemorrhages in sinusoidal spaces(S), cellular infiltration(CI). C. Standard group- showing well organized structure without any structural damage. D,E,F- Test groups 1,2 and 3- showing gradual recovery from structural abnormalities according to treated doses. Arrow marks indicated the structural damage of hepatic tissue.

Discussion

CPA is the well known anticancer drug used against different types of cancers. Along with therapeutic actions, CPA produces various side effects, in that hepatotoxicity is one of the main toxic effect to focus, because liver is the main organ to target by the chemicals and drugs for its metabolic process. During this process harmful substances and metabolites will generate, these may produces direct toxic effects on the liver(Xinsheng & Jose, 2012). So the current study was focussed on the protective effect of *Neptunia triquetra* extract on hepatotoxicity induced by the cyclophosphamide.

Hepatotoxicity induced by CPA treatment described by different parameters such as decreased final body weight, increased liver weight of rats and serum lipid profile parameters includes cholesterol, triglyceride, LDL-cholesterol levels were increased, HDL-cholesterol levels were decreased (Khaled, et al., 2021) and increased levels of serum biochemical markers like AST, ALT, ALP, LDH (Masha& Shivanandappa,2013), TB, DB and decreased levels of TP, ALB were observed. And hepatic tissue oxidant, antioxidant marker enzymes such as CAT, GSH, SOD, GPx, GST, GR (Shanmugarajan, et al., 2008) levels were decreased and LPO levels were increased. And finally decreased the hematological parameters includes Hb content, RBCs and WBCs (Lata, et al., 2014) along with histopathological changes in liver architecture involves hepatic necrosis, sinusoidal obstruction, cellular infiltration, fatty cell deposits. These all significantly altered levels of serum, hepatic tissue and hematological parameters along with histopathological observations proved the hepatotoxicity of CPA.

Rats administered with test doses(100, 200 and 400mg/kg) of NTME along with CPA shown good protection from hepatotoxicity of CPA and this was proven with significantly improvement in changed parameters namely increased final body weight, decreased liver weight of rats and decreased levels of cholesterol, triglycerides, LDL-cholesterol, increased levels of HDL-cholesterol and decreased AST, ALT, ALP, LDH, TB, DB levels, increased TP, ALB levels and CAT, GSH, SOD, GPx, GST, GR levels were increased,

LPO levels were decreased and increased Hb content, RBCs, WBCs along with improved histopathology of liver from structural damage.

The dose dependent significantly improved serum, hepatic tissue and hematological parameters along with histopathological changes confirmed the hepatoprotective effect of NTME on CPA induced hepatotoxicity. This hepatoprotective effect of NTME could be possible with its phytoconstituents of alkaloids, steroids, flavonoids, terpenoids, phenolic compounds.

Conclusion

The obtained data revealed the hepatoprotective effect of *Neptunia triquetra* extract against cyclophosphamide induced hepatotoxicity and it was dose dependent. This was evidenced by normalization of the altered biochemical and antioxidant parameters along with histopathological parameters and the test dose at 400mg/kg has shown more significant effect compared to the doses of 100, 200 mg/kg. However further investigations are required to explore its protective role and mechanism behind the activity of *N.triquetra* extract.

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Conflict of interest

Authors have no conflict of interest.

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