ORIGINAL ARTICLE

An Assay of Albino Rat Hepatocytes in Conjunction with the Berberis Vulgaris Root Extracts during Cyclophosphamide Induction

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ABSRTACT

Background: Cyclophosphamide is a well-known, powerful anti-cancer treatment of choice for various tumors.
Aim: To study, the protective effect of *Berberis Vulgaris* methanolic root extract on hepatocytes of rats during CYP-induction by histopathological aspect was studied.
Place of Study: Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore.
Study Design: Observational type of descriptive study
Methods: Total 24 adult healthy male albino rats divided in six groups with four rats in each group (n=4) weighing between 120-200g were contained in Animal House of The University of Lahore and permissible to saline and usual regime with measured settings of temperature 25±2 and regular photoperiod (12 hours dark and light) during the experimentation. Berberis vulgaris root extracts were prepared in 70% ethanol, filtered and concentrated to dry on rotary evaporator at 50°C and Cyclophosphamide was obtained from Pharmedic Laboratories (pvt, limited), with dose of 1000mg/kg and 80mg/kg respectively, prepared in water for injection.
Results: Histological examination of saline treated group showed normal structure while the negative control group showed necrosis of hepatocytes, vacuole generation, marked cellular swelling and presence of inflammatory cells. The rat liver in plant control group A and B showed normal architecture like saline treated control group with blood vessel congestion. Analysis of prophylactic group showed slight presence of hepatocyte necrosis, vacuole generation, and cellular swelling in recovery phase.
Conclusion: Prophylactic group with *Berberis vulgaris* root extracts 1000 mg/kg exhibited noticeable safety in contradiction of diminished liver functions and toxicity induced by Cyclophosphamide.
Keywords: Berberis vulgaris, Cyclophosphamide, Hepatotoxicity.

INTRODUCTION

Berberis vulgaris Linn (barberry), an herb in traditional medicine[1]. *Berberis vulgaris* a typical garden bush shares the same history as old as humanity. It is locally present in Europe and the British Isles and North America[2]. *Berberis vulgaris Linn also* called as Barberry belongs to the family *Berberidaceae* consisting of almost 15 genera and about 650 species. It is in abundance in the northern hemisphere temperate regions. It is commonly scattered over the foremost parts of temperate Asia, Europe, and Northern Africa as well as in northern areas of Iran. *Berberis vulgaris is* also known as European barberry, because of its importance as a European *Berberidaceae* representative[1,2].

All parts of *B. vulgaris* have been used in outmoded remedy to cure diarrhea, colitis, gastroenteritis, and hepatic disorders. Several alkaloid constituents with an isoquinolinic nucleus, such as berberine, berbamine and palmatine were isolated[3]. Other compounds like terpenoidslupeol, oleanolic acid, stigmasterol and stigmasterolglucoside[4] as well as polyphenols[5] were also identified. Nevertheless, berberine is the most important alkaloid that is commonly appealed to be accountable for their advantageous possessions[6]. There are multiple pharmacological effects of berberine, such as antimicrobia[6,7], anti-tumor[8,9,10] and anti-inflammatory effects[11,12,13]. It also has effects on the gastro-intestinal[4,14,15,16], cardiovascular[17,18,19] &nervous systems[20].

Cyclophosphamide (CP), an oxazophosphorine-alkylating agent, is broadly used as an antineoplastic drug in chemotherapeutic treatments of lymphoproliferative ailments, solid lumps and as an immunosuppressant in the conduct of autoimmune ailments like nephritic syndrome, systemic lupus erythematosus and rheumatoid arthritis[21]. In addition, CP is of paramount importance as an immunosuppressive agent in organs and bone marrow transplant regimens[22]. From this, CP is used clinically to treat a wide range of cancers including malignant lymphomas, myeloma, leukemia, mycosis fungoides, neuroblastoma, adenocarcinoma, and breast carcinoma[22,23]. Other clinical uses for CP include immunosuppressive therapy follows organ transplants or as a treatment for autoimmune disorders such as nephritic syndrome, Wegener's granulomatosis and rheumatoid arthritis[24]. A well-known exemplified literature implies that raised therapeutic dose of cyclophosphamide, may cause the hepatic disorders due the development of total serum bilirubin level and sinusoidal obstruction syndrome[25].

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MATERIALS AND METHODS

The research etiquette was permitted by Ethics committee of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore. 24 grown-ups, in good physical shape male albino rats considering amongst 120–200g were included in the existing study. The animals were separated into six groups having 04 rats in separate groups (n=4). Total experimental duration was of 13 days and dissection was done on 7th and 13th day. Dosing time was 02:00pm. Roots of *Berberis vulgaris* were used and Cyclophosphamide was a product of Pharmedic Laboratories (pvt, limited). Dose of Cyclophosphamide (80mg/kg), while *Berberis vulgaris* dose (1000 mg/kg) was prepared in distilled water. Cyclophosphamide was injected via intraperitoneal route and Berberis vulgaris given orally via gastric intubation. Samples from liver were removed and preserved in 10% buffered formalin for histopathology later. It was performed by cutting transverse sections of liver tissues and then stained. Other parts of Liver samples were stored at -80 C for further testing. Dose of Cyclophosphamide (80 mg/kg), while *Berberis vulgaris* dose (1000mg/kg) was prepared in distilled water. Cyclophosphamide was injected via intraperitoneal route and Berberis vulgaris given orally via gastric intubation. Samples from liver were removed and preserved in 10% buffered formalin for histopathology later. It was performed by cutting transverse sections of liver tissues and then stained. Other parts of Liver samples were stored at -80 C for further testing. The specimens were fixed in Bouin's fluid for 02 hours, and then dehydrated in ascending grades of alcohol (70, 95, and 100%), cleared in xylene, and then embedded into paraffin wax. Histological sections of 3–5µm thickness were prepared from the paraffin blocks. The paraffin sections were stained with different histological and in the Pathology Department, Avicenna Medical College & Hospital, Lahore. Histological stain used in this study was as follows:

(1) Hematoxylin and Eosin (H&E) or Masson's trichome stains.

RESULTS

Table 1: Study Design: -

Groups	Dose	Dissected on
Saline treated control group	Rats were given daily with 0.9% NaCl for 12 days.	13th day
Negative control group	The animals received 80mg/kg cyclophosphamide alone for 6 days Intraperitoneally (i.p) to induce toxicity.	7th day
Plant control group A	The animals received 1000 mg/Kg of Berberis extract alone orally for 6 days.	7th day
Plant control group B	The animals received 1000 mg/Kg of Berberis extract alone orally for 6 days.	13th day
Combination group	The animals received both 80mg/kg cyclophosphamide i.p and 1000mg/kg *B.vulgaris* extract orally for 6 days.	7th day
Prophylactic group	Rats in this group were given *B.vulgaris* extract 1000mg/kg orally for 6 days and then received cyclophosphamide i.p. 80mg/kg for next 6 days.	13th day

Table 2: Histopathological liver findings:

Groups	Hepatocyte Necrosis	Vacuole Generation	Inflammatory Cells	Fatty Changes	Microvascular Steatosis	Cellular Swelling
Saline Control	-	-	-	-	-	-
Negative control	++	+	++	+	+	+++
Plant Control A	-	+	-	+	-	
Plant Control B	-	+	-	+	-	+++
Combination	++	-	++	-	++	+++
Prophylactic	+	+	+	+	-	+

- = not present ++ = present, + = slightly present +++ = strongly present

Table 3: Analysis of variance of Liver Function Test including ALT (U/L), AST (U/L), Alkaline Phosphatase (U/L), Total Proteins (g/dL) and Serum Albumin (g/dL) Levels in control group, Berberis Control group, Negative Control and groups treated with Berberis vulgaris in combination and before cyclophosphamide.

Groups VS Hepatic Makers			Sum of Squares	df	Mean Square	F	Sig.
Serum ALT	Between Groups	(Combined)	129730.00	5	25946.00	25.61	.000
	Within Groups		18236.00	18	1013.11		
	Total		147966.00	32			
Serum AST	Between Groups	(Combined)	107855.70	5	21571.14	3.504	.022
	Within Groups		110823.250	18	6156.847		
	Total		218678.958	23			
Serum Alkaline Phosphatase	Between Groups	(Combined)	404419.500	5	80883.900	16.900	.000
	Within Groups		86147.000	18	4785.944		
	Total		490566.500	23			
Serum Total Protein	Between Groups	(Combined)	.708	5	.142	3.806	.016
	Within Groups		.670	18	.037		
	Total		1.378	23			
Serum Albumin	Between Groups	(Combined)	5.338	5	1.068	65.146	.000
	Within Groups		.295	18	.016		
	Total		5.633	23			

* = Significant as p<0.05

According to analysis of variance p value comes out to be less than 0.05 depicting significant difference of mean ALT levels between all groups as compared to control groups. Furthermore, analysis of variance overall significant difference in mean value of all groups. Serum Total Proteins and Serum Albumin level was observed among all the groups as p <0.05.

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Fig 1: Section of Normal Saline Treated Control Rat liver- Histological analysis of the control group rats' liver revealed no noteworthy eccentricityas of standard architecture. Typical histological manner of hepatocytes was pragmatic in control group rats and the portion marked by arrow head is position of central vein.

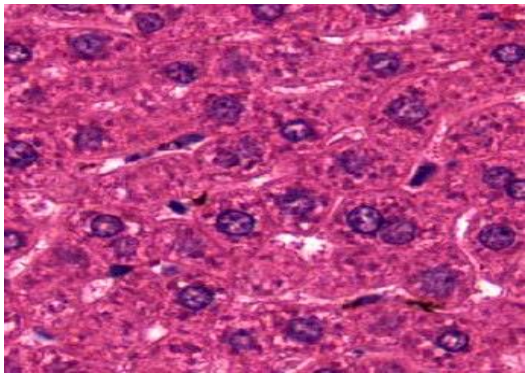
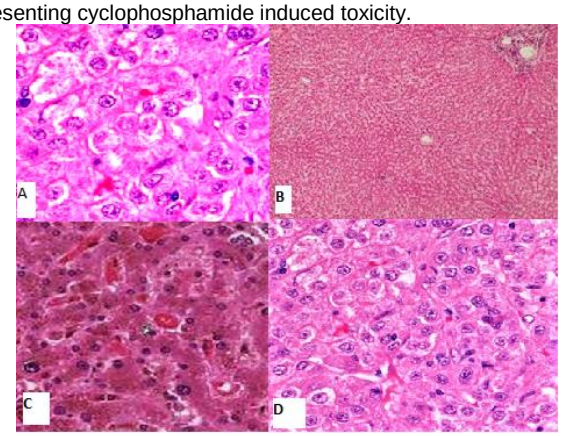


Fig 2: (A) Section of Negative Control Group Rat – Liver The liver histological structure of the negative control group rat was significantly affected by cyclophosphamide administration presenting cyclophosphamide induced toxicity.



Total destruction of hepatocytes with disarray of architecture of hepatocytes was observed in negative control group as compared to control group hepatic section architecture. Vacuole generation along with inflammatory cells infiltration was also observed in portal areas. Fatty changes and microvascular steatosis was found. In negative control group histological changes were observed with hepatocyte swelling and degeneration. **(B)** Section of Plant Control Group Rat liver- Histological examination of the rat liver in plant control group showed normal architecture as of control group. Slight fatty changes were also observed as well as vacuole generation. **(C)** Section of combination group rat liver- Histological analysis of combination group revealed markedhepatocyte swelling and necrosis. Inflammatory cells infiltration was also present along with vacuole generation. **(D)** Section of Prophylactic Group Rat liver - Histological analysis of prophylactic group revealed initiation of noteworthy regenerative fluctuations. Added unvarying placement of hepatocytes was detected in prophylactic group as equated to Negative control group.

DISCUSSION

The present investigation revealed that the liver of the control group (G1) revealed normal hepatic architecture; hepatic parenchyma, hepatic lobulation, hepatic cord, hepatic portal triad, hepatocytes and hepatic sinusoids. Meanwhile, Negative control group and Combination group (initially treated with cyclophosphamide for 6 days) treated with Cyclophosphamide in a dose of 80 mg/kg. but showed moderate to severe steatosis and necrosis with moderate disorganization of hepatic cords. In addition, multiple and focal scattered of inflammatory cells infiltration with fibrous connective tissue proliferation was demonstrated within the hepatic tissues especially portal areas. Furthermore, moderate sinusoidal dilatation in between the hepatic cords and hepatocytes glycogen depletion were also observed.

The present study illustrated thatdiffuse degeneration and necrosis of hepatic tissues with loss of the hepatic architectures were clearly observed. This result is in parallelism with[26] who described that cyclophosphamide induced hepatocytes necrosis and this finding may designate current hepatotoxicity grievance occasioned from the termination of protein blend owing to overpowering to these nanoparticles. Necrosis is encouraged by toxicants that occurrence the cell organelles particularly the mitochondria, endoplasmic reticulum and nucleus thus alarming their activity. Severe fibrous tissue proliferation with anti-inflammatory cells infiltration; plasma cells, mast cells, lymphocytes and eosinophils were observed within the hepatic parenchyma especially portal triad. Hexagonal lobules are centered on the central vein that exhibited moderate to severe congestion with the hepatic artery, sinusoids and Portal vein. These investigations are coinciding with[27,28] who claimed that the histological analysis of liver sections presented zones of necrosis convoyed by noteworthy stirring cell permeation near by the hepatic portal triad at day 07 of experience. By this time, the engulfed Cyclophosphamide would have dissolved, releasing mustard compounds, which may have caused toxicity to the surrounding hepatocytes as well.

Furthermore, disorganization of hepatic cords was observed. In addition, severe degenerative changes which were evident in numerous hepatocytes; enlarged cells, had light and foamy cytoplasm filled with vacuoles of variable size that were tended to form cystic degeneration were claimed. Hepatocytes necrotic changes were evident; a small, pyknotic cellular nuclei with condensed chromatin, lack of nucleolus and acidophilic cytoplasm were observed were recognized. And also, hepatocytes cytoplasmic vacuolation with partial cytoplasmic swelling was well demonstrated. Moreover, several forms of nuclear abnormality were exhibited;binucleation, nuclear

vesiculation, anisokaryosis, karyolysis and nuclear membrane irregularity and apoptosis.

The perceived apoptosis in the liver of rats pickled with Cyclophosphamide might be caused from intercellular traumaencouraged by these acceptableelements [29]. Apoptosis might be tracked by mitochondrial inflammation, endoplasmic reticulum dilatation and lysosomal disagreementformerlylessening and termination of nuclei[30].

CONCLUSION

The outcomes of the present study inferred that experimental administration of Cyclophosphamide at the dose of 80 mg/kg was accompanied by marked derangement of structure of hepatocytes in rats as evidenced by histopathology and biochemical analysis. The methanolic root extract of *Berberis vulgaris* can play a significant protective role in prophylactic treatment against Cyclophosphamide-induced effects in liver. However, pretreatment with *Berberis vulgaris* extract exhibited more significant protective activities against liver cells as compared to combination group (Cyclophosphamide and *Berberis vulgaris* given simultaneously).

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