ORIGINAL ARTICLE

Sequels of Methanolic *Berberis Vulgaris* extract in Cyclophosphamide induced hepatotoxicity in Rats

HAROON HABIB, YASIR ALI BHATTI, KAMRAN ARZOO, MUZAMIL LIAQAT ALI, ALI IFTIKHAR, MUDASER HUSSAIN ABBASI, USAMA ISHTIAQ,MUSHTAQ FAREED

[1]*Assistant Professor Biochemistry, Avicenna Medical College, Lahore*
[2]*Assistant Professor Biochemistry, Avicenna Medical College, Lahore*
[3]*Assistant Professor Physiology, Rai Medical College, Sargodha,*
[4]*Assistant Professor Biochemistry, Avicenna Medical College, Lahore*
[5]*Assistant Professor Biochemistry, Avicenna Medical College, Lahore*
[6]*Professor, Forensic Medicine and Toxicology, Rai Medical College, Sargodha*
[7,8]*Molecular Biologists, Vital Molecular Diagnostics and Research Laboratory, Lahore.*
Correspondence to Dr. Muzamil Liaqat Ali, Email: mhaabbasi79@gmail.com

ABSRTACT

Background: Cyclophosphamide (CP) is branded as an effective anti-cancer medication with hostile effects including induced hepatotoxicity, nephrotoxicity, cardiotoxicity and elevated cholesterol levels along with marked hematological effects. In this article, the protecting effects of *Berberis vulgaris* methanolic root extracts (Pre & Post treatment) on CP-induced ALT, AST variations in rats were calculated.
Place of Study: Study was piloted in Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore.
Duration of Study: 01 year from 01 January 2015 - 31 December 2015
Study Design: Observational type of descriptive study
Methods/Results: Total 24 adult healthy male albino rats divided in six groups with four rats in each group (n=4) weighing between 120-200g were housed in Animal House of The University of Lahore and permitted entree to saline and standard diet under measured settings of temperature 25±2 and photoperiod (12 hours dark and light) allowed, during the trial. *Berberis vulgaris* root extracts were prepared in 70% ethanol, filtered and concentrated to dry on rotary evaporator at 50°C and Cyclophosphamide was procured from Pharmedic Laboratories (pvt, limited), with dose of 1000mg/kg and 80mg/kg respectively, prepared in water for injection. The experimental rats were anesthetized by chloroform and then dissected. Blood was collected directly from Heart, then centrifuged right after and serum was separated for complete biochemical analysis, and aseptic measures were taken. For further use, -80°C was the temperate of tissue samples of liver, kidney, heart and brain storage in 10% buffered formalin.
Conclusion: It was determined that, *B. vulgaris* has probable ameliorative role in deterrence of onset and progression of cyclophosphamide-induced hepatotoxicity.
Keywords: Berberis vulgaris, Cyclophosphamide

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].

Multiple ailments like gastroenteritis, colitis, diarrhea and liver issues are treated with traditional medications from fruit, leaf, bark and root extracts, consist of diverse range of alkaloid isolates; palmatine, berbamine and berberine[3] along with oleanolic acid, terpenoidslupeol, stigmasteroglucoside[4] and polyphenols[5], appealed to partake advantageous properties[6] including antimicrobial[6,7], anti-tumor[8,9,10] and anti-inflammatory[11,12,13], gastrointestinal[4,14,15,16], cardiovascular[17,18, 19] and nervous systems[20] as well as broadly used in diverse chemotherapeutic regimens as antineoplastic agent in various lymphoproliferative and immunosuppressant in autoimmune disease like rheumatoid arthritis, systemic lupus erythematosus and nephritic syndrome21). Moreover, CP possesses supreme standing in organs and bone marrow transplant regimens as immunosuppressive mediator[22] and for this very reason used clinically in treatment of breast carcinoma, adenocarcinoma, neuroblastoma, mycosis fungoides, leukemia, myeloma, malignant lymphomas[23] as well as wegnener's syndrome[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

Present study was steered at "The University of Lahore" in "Institute of Molecular Biology and Biotechnology" (IMBB). Roots of Berberis vulgaris were used and Cyclophosphamide was a product of Pharmedic Laboratories (Pvt, Ltd). Male, adult and healthy albino rats (total=24), weighing approx. between 120-200g were

inducted and divided in six groups with four rats in apiece group (n=6). Total experimental duration was of 13 days and dissection was done on 7th and 13th day. Dosing time was 02:00pm. Dose of Cyclophosphamide (80 mg/kg. body weight), while Berberis vulgaris dose (1000 mg/kg. body weight) was prepared in distilled water. Cyclophosphamide was injected via intraperitoneal route and Berberis vulgaris given orally via gastric intubation. The blood was directly collected from the heart of each rat through heart puncture under the chloroform anesthesia. Blood taken was subjected to centrifugation in order to separate serum within one hour after collection of blood. The serum samples analyzed for estimation of alanine transaminase (ALT) and aspartate aminotransferase (AST).

Tissues samples of liver were obtained and well-preserved in 10% buffered formalin at -80°C for further analysis. *Berberis vulgaris* roots were gathered from Swat, Pakistan, dried out at temperature 25±2 for two weeks and by crushing and dipping 100 gm of material in 500 ml of 70% methanol in a well-plugged glass vessel extract was set, and kept away from direct exposure of sunlight and swirled twice a days for 5 minutes manually. After 2 weeks liquid was filtered and concentrated to dryness on rotary evaporator at 45℃. Mass of the dry solid obtained was 12.42 gm. Study was divided in to six groups including the saline treated control group in which rats were given daily with 0.9% NaCl for 12 days and dissected at 13th day second group was Negative control group in which animals received 80mg/kg cyclophosphamide alone for 6 days Intraperitoneally (i.p) to induce toxicity and dissected at 7th day. Third group was Plant control group A in which animals received 1000 mg/Kg of Berberis extract alone orally for 6 days and dissected on 7th day. Fourth group was Plant control group B in which the animals received 1000 mg/Kg of Berberis extract alone orally for 6 days and dissected on 13th day. Fifth group was combination group in which animals received both 80mg/kg cyclophosphamide i.p and 1000mg/kg *B.vulgaris* extract orally for 6 days and dissected on 7th day, and last sixth group in which the 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 and dissected on 13th day.

RESULTS

Statistical software package SPSS version 22.0 for windows used for statistical analysis. The obtained data was entered on SPSS 22.0. Furthermore, the tables and graphs showing the results were also generated on SPSS 22.0 and Microsoft Excel 2013. The comparison of parameters values between experimental and control groups was performed by with one-way ANOVA and independent samples t- test. Values were considered, statistically significant when $p < 0.05$. Whereas, Figure 1 showed descriptive statistics (Mean & SD) of liver function test including ALT (U/L), AST (U/L), Levels in control group, groups suffering from cyclophosphamide induced toxicity, Berberis group and groups treated with Berberis vulgaris before and in combination with cyclophosphamide- induced toxicity. The mean value of ALT in control group was recorded to be 155.25±27.94. The negative control group administered with Cyclophosphamide for 6 days, the mean value was 330.75±9.56 which was considerably higher than the control group. The increase was greater for group dissected after 12 days. The groups that were administered with *Berberis vulgaris* mean value i.e., plant control A and B mean ALT levels were 92.75±24.03 and 158.50 which were very close to average value of control group. In group treated with both cyclophosphamide and *B.vulgaris* simultaneously (combination group) the mean level was 206.75±63.30 while in prophylactic groups' average value of ALT was 184.00±80.20. ALT levels of control and *Berberis vulgaris* groups were almost same with non-significant difference between the two. Increased levels of ALT were found in negative control group treated with Cyclophosphamide only which is the marker of disturbed liver function. The increase was less for plant control group A dissected after 6 days as compared to plant control group B dissected after 12 days revealing greater toxicity induced by cyclophosphamide with increase in time. The average ALT levels for combination group were found to increase in comparison to control group. The ALT levels were almost same in prophylactic group depicting the therapeutic effect of *B.vulgaris* against cyclophosphamide toxicity. The mean value of AST in control group was recorded to be 318±50. The negative control group administered with cyclophosphamide for 6 days, the mean value was 347.75±10.78. The groups that were administered with *Berberis vulgaris* mean value i.e plant control A and B mean AST levels were 242.75±17 and 367.25±142.36 which were very close to average value of control group. In group treated with both cyclophosphamide and *B.vulgaris* simultaneously (Combination Group) the mean level was 187.00±113.33 while in prophylactic groups average value of AST was 223.00±39.80. No significant difference between control and *B.vulgaris* Plant control group A was observed. Increased level of AST was found in cyclophosphamide Negative Control group revealing that marked hepatotoxicity was induced by cyclophosphamide in that group. Average value of Combination group was found below as that of Control group with no significant difference between the two while average value of prophylactic group showed considerable reduction of cyclophosphamide induced toxicity depicted by lowering of AST level. So, it was evident from the AST result that therapeutic effects of *B.vulgaris* markedly reduced the increased AST levels near to normal by showing healing properties against cyclophosphamide-induced liver toxicity. Table 1 showed the results 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 including $p < 0.05$.

Figure 1: Mean values of Liver Function Test including ALT (U/L), AST (U/L), in control group, groups suffering from cyclophosphamide induced toxicity, Berberis group and groups treated with *Berberis vulgaris* before and in combination with cyclophosphamide-induced toxicity.

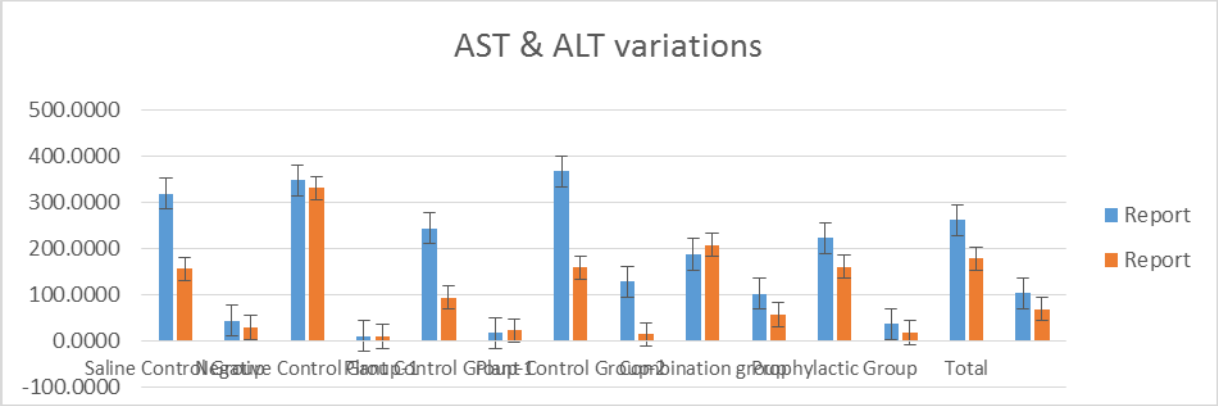

Table 1: Analysis of variance of Liver Function Test including ALT (U/L), AST (U/L) Levels in control group, berberis control group, negative control and groups treated with *Berberis vulgaris* in combination and before cyclophosphamide

ANOVA Table

		Sum of Squares	df	Mean Square	F	Sig.
Serum AST Level * Groups	Between Groups (Combined)	402225.238	5	80445.048	13.255	.000
	Within Groups	473379.750	78	6068.971		
	Total	875604.988	83			
Serum ALT Level * Groups	Between Groups (Combined)	306288.452	5	61257.690	61.317	.000
	Within Groups	77924.250	78	999.029		
	Total	384212.702	83			

* = Significant p<0.05.

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DISCUSSION

The foremost function of chemotherapeutic drugs, is to destroy rapidly proliferating normal and cancer cells, which may include RBCs (26). CP, an alkylating mediator imparts its function by forming cross-linkages with DNA of the tumor, metabolized in liver to active structure and eliminated primarily via kidneys. The liver is at risk to be damaged due to direct experience to noxious products owing to its role in detoxification of xenobiotics and metabolic by-products. The raise in liver enzymes activities are owing to liver dysfunction with a consequential drop in their biosynthesis and rehabilitated membrane permeability permitting enzyme seepages into serum[27].

The highpoint observation cleared in this study that massive exposure to cyclophosphamide led to raised liver toxicity including high levels of serum ALT and AST if compared with normal control rats, and mortality as well. Synchronized administration of berberine and CP fashioned a probable mitigation of changed serum ALT and AST levels, despite the fact concentration was declined in CP-administered rats and was meaningfully amplified in group simultaneously received berberine and CP (both). From many pre-historic cultures, it is evident that toxicities are treated with curative herbs and they play a pivotal role in treatment of chief health issues because of their preventive actions all over the sphere. Almost one-fourth of recommended drugs made from the extracts of plants or their effective components[28].

One of the major medicinal plant used for its therapeutic properties is *Berberis vulgaris* (family Berberidaceae) and aim of this research work is to establish the reported curing effects of *B.vulgaris* root extracts against cyclophosphamide-induced hepatological toxicity. In the present study comparison of pre-treatment and post-treatment was also observed in order to find that which treatment (either pre or post treatment) accelerated more recovery. Recent studies revealed that Berberis vulgaris have several therapeutic components with strong antioxidant effects such as berberine. As it was reported from the recent studies that methanolic root extract of Berberis vulgaris has marked antioxidant properties (29). So, in the present work methanolic extract of Berberis vulgaris root was used to overcome the hematological and hepatological toxicity produced by cyclophosphamide.

CONCLUSION

This research work and findings intended to conclude the fact that *B.vulgaris* has likely benefits in deterrence of beginning and progression of CP-induced Hepatotoxicity.

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