ORIGINAL ARTICLE

Invigoration Observed by the use of Methanolic *Berberis Vulgaris* Root Extract in Cyclophosphamide-induced Cardiotoxicity in rats

YASIR ALI BHATTI[1], HAROON HABIB[2], ADNAN BASHIR[3], *MUZAMIL LIAQAT ALI[4], ALI IFTIKHAR[5], MUDASER HUSSAIN ABBASI[6], ARIF MALIK[7]*

[1,2,4,5]Assistant Professor Biochemistry, Avicenna Medical College, Lahore

[3]Assistant Professor Pharmacology, Fatima Memorial College of Medicine & Dentistry Shadman, Lahore[3]

[6]Professor, Forensic Medicine and Toxicology, Rai Medical College, Sarghoda[6]

[7] Professor IMBB, University of Lahore

Correspondence to Dr. Mudaser Hussain Abbasi, Email: forensicdoctor786@gmail.com, Cell: 0336-2234937

ABSRTACT

Background: Being branded as an effective anti-cancer medication, Cyclophosphamide induces some well-established grave effects such as elevated cholesterol levels, marked hematological effects, along with nephrotoxicity, hepatotoxicity, cardiotoxicity, along with abnormal values of the serum LDH and the serum AST levels.

Place of Study: This study was supervised by Prof. Dr. Arif Malik and conducted in the Institute of Molecular Biology and Biotechnology (IMBB) of the University of Lahore, Lahore.

Duration of Study: The total duration that this reported study took was about one year starting on January 1, 2015 and concluding on December 31, 2015.

Study Design: After due consideration and deliberation, an observational type of descriptive study was designed.

Methods/Results: During the era of this study, a total of 24 healthy male adult albino rats were segregated in six groups of four rats each (n = 4) while they were housed in Animal House of the University of Lahore, Lahore. Each rat weighed about 120 - 200 grams. The rats were fed with standard diet with saline and they were kept under measured settings of 25 ± 2^{o}C along with a photoperiod of 12 hours dark and 12 hours of light. The 70% ethanol was used to prepare the *Berberis vulgaris* root extracts, then the preparation was filtered and concentrated by drying the filtrate on the rotary evaporator at 50^{o}C. Pharmedic Laboratories (PVT, Limited) was employed to procure the drug Cyclophosphamide. *Berberis vulgaris* root extracts and CP was given to the rats at the dose of 1g/Kg body weight and80 mg/Kg body weight respectively. After procurement, Cyclophosphamide was prepared to be used in and injection from by using distilled water.

Conclusion: An ameliorative role of the *Berberis vulgaris* was observed in deterrence of onset and progression of cyclophosphamide-induced cardiotoxicity.

Keywords: Berberis vulgaris, Cyclophosphamide (CP), Cardiotoxicity

INTRODUCTION

Sharing the historical background as old as the human history itself, the *Berberis vulgaris Linn* (barberry) is a typical garden bush found primarily in the British Isles and North America and Europe and abundantly in temperate regions of northern hemisphere including the temperate regions of Asia, Northern Africa and northern areas of Iran. Belonging to the family *Berberidaceae,* spanning over 15 various genera and almost 650 diverse species, barberry is also known as the European barberry[1,2].

Various ailments like hepatic disorders, gastroenteritis, colitis and diarrhea have been treated in traditional medicine with the utilization of fruit, leaf, bark and root of barberry. Possessing an isoquinolinic nucleus, a diverse range of alkaloid constituents (berberine, berbamine & palmatine) had already been isolated in previous studies[3]. Some other such studies had also reported isolation of constituents like oleanolic acid, terpenoidslupeol, stigmasterol & stigmasterol-glucoside as well as polyphenols[4,5].

Out of all the foresaid alkaloids and other alkaloids as well, the most important one is claimed to be the berberine being responsible for its beneficial effects[6]. Antimicrobial+, anti-tumor[8,9,10] and anti-inflammatory effects[11,12,13] are some of the wide array of the pharmacological effects berberine.

Cyclophosphamide (CP) is an oxazophosphorine-alkylating agent that is commonly utilized as an antineoplastic drug in chemotherapeutic regimens of lymphoproliferative disorders, certain solid tumors. It is also employed as an immunosuppressant in the treatment of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE) and nephritic syndrome[21]. The importance of CP as an immunosuppressive agent in organs and bone marrow transplant regimens is presumed to be second to none[22].

Various clinical trials had already established the clinical significance of CP in the management of a diverse range of cancers comprising of mycosis fungoides, neuroblastoma, adenocarcinoma, CA breast, myeloma, leukemia and malignant lymphomas[22,23]. Autoimmune disorders such as Wegener's granulomatosis, rheumatoid arthritis and nephritic syndrome are very handsomely managed by the immunosuppressive attributes of CP[24].

MATERIALS AND METHODS

The present study was supervised and conducted in the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore. Pharmedic Laboratories

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(Pvt) Ltd was employed to procure the Cyclophosphamide. Moreover, the roots of the *Berberis vulgaris* were also used. About a couple of dozens of adult male albino rats were selected after making sure that they were in healthy state weighing about 120 – 200 grams each. The selected animals were divided in to six groups of four rats each (n=4).

Berberis vulgaris roots were collected from the valley of Swat, which is located in the Northern areas of Pakistan. After collection, these roots were air dried at room temperature for a couple of weeks. Once the roots were dried, they were crushed. About a hundred grams of the dried and crushed roots in about half a liter of 70% methanol solution in a properly capped glass containers. These containers were kept away from the direct sunlight in a well shaded area at room temperature. These containers were swirled around twice a day manually for about five minutes every time for a couple of weeks. After the two weeks were over, the contents of the glass containers were filtered out and the filtrate was concentrated by drying it on rotary evaporator at 45^{o}C. After the whole procedure was done, a mass of dry solid of 12.42 grams was obtained.

The drugs, Cyclophosphamide and *Berberis vulgaris*, were given to the rats daily during the course of the experiment at 2 P.M. Both of the drugs, Cyclophosphamide and *Berberis vulgaris*, were prepared by in distilled water. Cyclophosphamide was injected to the rats by using the intraperitoneal route at the dose of 80 mg/Kg body weight. Whereas, *Berberis vulgaris* was given orally to the rats at the dose of 1 g/Kg body weight.

The total duration of the experiment was set to be thirteen days, whereas, the dissection of the animals was scheduled in the seventh and the thirteenth day. On the dissection day, the rat to be dissected was administered the chloroform as an anesthetic and the dissection was carried out when the animal was knocked out. Once the animals were knocked out, blood was drawn directly from the heart of each rat by puncturing the heart musculature. After the blood sample was drawn, it was centrifuged so that the serum could be separated, and this was carried out within an hour of collection of the sample. Later the serum samples were analyzed in the lab to assess the levels of Lactate dehydrogenase (LDH) and Aspartate Aminotransferase (AST). Once the blood samples were drawn from the rats, the tissue samples of the heart collected and they were right away preserved in the 10% buffered formalin solution. These preserved tissue samples were stored at –80^{o}C to analyze them histopathologically later.

GROUPS:

Control group - administered with normal diet and water and dissected on 13th day.

Negative control group - the animals received 80mg/kg cyclophosphamide alone for 6 days intraperitoneal (i.p) to induce toxicity and dissected on 7th day.

Plant control group-A - the animals received 1000 mg/Kg of Berberis extract alone orally for 6 days and dissected on 7th day.

Plant control group-B - the animals received 1000 mg/Kg of Berberis extract alone orally for 6 days and dissected on 13th day.

Combination group:- The animals received both 80mg/kg cyclophosphamide i.p and 1000mg/kg B.vulgaris extract orally for 6 days and dissected on 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 and dissected on 13th day.

RESULTS

The collected data was subjected to the statistical analysis by SPSS Version 22.0. The table and the graphs published in this study were also generated by employing the same software, SPSS 22.0, moreover Microsoft Excel 2013 was also used. One way ANOVA and Independent sample T-test were used to compare the parameter values between the experimental and the control groups. As a result, a statistically significant $p < 0.05$ was observed.

While analyzing the control group, the mean value of AST was observed to be 314.7±39.8, whereas, the mean value of AST was calculated to be 340.0±10.0AST in the negative control group – A which was given CP for six days. Contrarily the mean values of AST in the Plant group A & B, the groups that were administered with the *Berberis vulgaris*, were analyzed to be 234.7±15.5 and 362.0±132.70 respectively.

The values observed in the Plant Control group A & B were analyzed to very close to the average value observed in the Control group. Lastly the mean values of AST in the Combination group, the group that was treated with both *Berberis vulgaris* and CP simultaneously, was analyzed to be 187.00±113.33, whereas, the mean values of AST in the Prophylactic groups was analyzed to be 221.0±39.8.When the Control group & the *Berberis vulgaris* Plant group A were compared, there was no significant difference that could be observed and reported. Although an increased level of the AST was observed in the CP Negative group B depicting that there was a remarkable CP-induced cardiotoxicity in the animals of this group. Moreover, there was an even higher level of AST that was observed in the Negative Control group that was dissected after the span of 12 days, i.e. on the 13th day. The average values of the Control group and that of the Combination group showed no significant difference, but on the other hand a considerable reduction of CP-induced cardiotoxicity was observed in the Prophylactic group and this reduction in the toxicity was evident by observing a marked decrease in the AST levels of the animals in this group. As far as the analysis of the AST levels are concerned, it can surely be reported that *Berberis vulgaris* possesses healing and protective effects against the CP induced cardiotoxicity, shown primarily by the decreased levels of AST close to the normal values of the Serum AST.

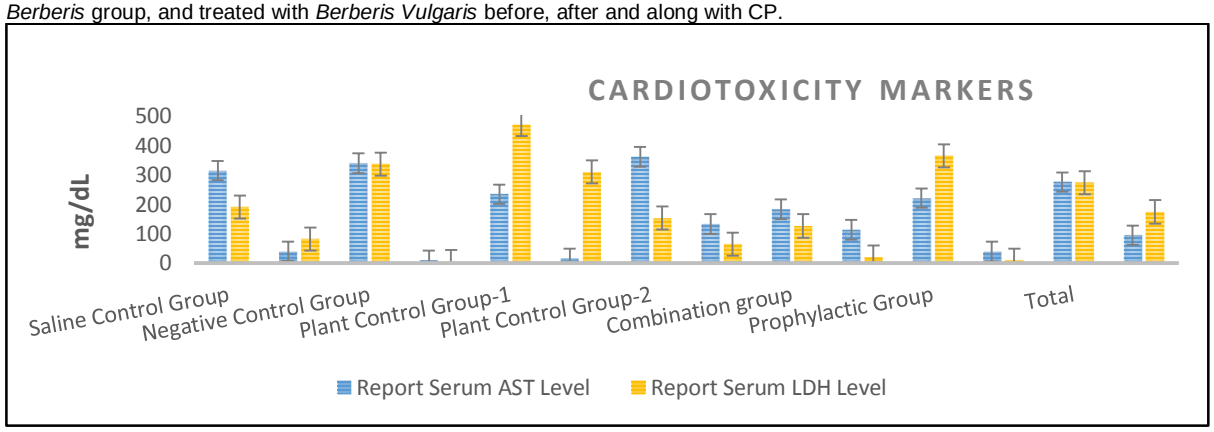
The mean value of the serum LDH in the Control group was analyzed to be 191.50±82.76, whereas, almost double these values of the serum LDH were observed in the Negative Control group – A, that was given CP for six days, i.e. 337.50±5.19. Additionally, the mean values of the serum LDH levels in the Plant Control group A & B, the groups that were treated with *Berberis vulgaris*, were analyzed to be 470.75±309.60 and 152.75±64.9. The serum LDH levels of the subjects of the Negative Control

group A & B revealed a significantly marked increase in the serum LDH levels, contrarily, a significantly marked decrease was revealed in the mean values of the serum LDH levels of the subjects of the Combination group, i.e. 126.50±20.15, pointing towards the deduction that the *Berberis vulgaris* showed protective and healing effects on CP induced damage and cell necrosis. Lastly, the mean values of the serum LDH level in the subjects of the Prophylactic group was analyzed to be 365.5±11.0.

Table 2: The values of mean and standard deviation of the serum AST and serum LDH levels among various groups such as cyclophosphamide-induced toxicity, Berberis group and the groups treated with *Berberis vulgaris* after, before and in combination with cyclophosphamide- induced toxicity.

Groups	Serum AST (Mean±S.D)	Serum LDH (Mean±S.D)
Saline Control Group	314.7±39.8	191.5±82.7
Negative Control Group	340.0±10.0	337.50±5.1
Plant Control Group-A	234.7±15.5	470.7±309.6
Plant Control Group-B	362.0±132.7	152.7±64.9
Combination group	183.0±113.3	126.5±20.1
Prophylactic Group	221.0±39.8	365.5±11.0

Figure 1: Variations in the mean values of serum AST (mg/dL) & serum LDH (mg/dL) levels in Control group, with CP induced toxicity, *Berberis* group, and treated with *Berberis Vulgaris* before, after and along with CP.



DISCUSSION

A cardiac dysfunction is an important cause of high morbidity and mortality probably resulting due to induction by chemotherapeutic agents. In addition, the inference of multiple chemical drug regimens with higher dosage leads to more profound cardiotoxicity as a potential complication. There is an incidence of 2-17% of lethal cardiomyopathy with subject to exposure of different population as well as their regions.

Cyclophosphamide induced cardiac toxicity take place within 2-3 weeks following treatment whereas more prominent chemical "Anthracyclines" for similar pathology take up to several months or even years with very high doses.

The studies reveal that Berberine is capable of protecting against the Cyclophosphamide-induced cardiotoxicity in animal model cases. A clinical dose of (80mg/kg) of Cyclophosphamide led to compromised cardiac functions, accentuated by the rise in serum AST and LDH levels. Simultaneously, it also resulted in decreased serum albumin levels. Berberine as a therapeutic agent when administered in pretreatment cases showed remarkable decrease in incidence of cardiotoxicity.

Thus, a recommendation can be drawn due to fact that berberine may have a protective role in Cyclophosphamide -induced Cardiotoxicity in addition to its existence as a potential factor in reduction and anticipation of the terminally complicated cases of cardiac origin in health providing setups. Cyclophosphamide has been recognized as an extremely robust and efficient anti-cancer mediator, so in the current study methanolic root extract of *Berberis vulgaris* was used to overcome toxicity produced as a consequence to cyclophosphamide.

CONCLUSION

Our findings highlight important aspects regarding potential benefits of *Berberis vulgaris* in prevention of onset and progression of cardiotoxicity induced as a result of Cyclophosphamide.

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