ORIGINAL ARTICLE

Effects of *Berberis vulgaris* root extract supplementation on Hemoglobin (Hb), Platelets and Total Leucocytes Count Variations among rats suffering from Cyclophosphamide-induced Hepatotoxicity: a retrospective follow-up study

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ABSTRACT

Background: In the present study twenty four (24) adult male healthy albino rats were used. Each rat weighed about 120-200g. The rats were obtained from the Animal House of the University of Lahore, Lahore. The rats were given cyclophosphamide that induced hepatotoxicity. Berberis vulgaris was given to the rats undergone cyclophosphamide induced hepatotoxicity and the encouraging results were observed.

Aim: To assess change in Hemoglobin (Hb), Platelets and TLC among cyclophosphamide induced hepatotoxicity affected rats with and without *Berberis vulgaris* supplementation (Pre and Post treatment).

Place of Study: Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore.

Duration of Study: The span of the study was about one year, from 1st January 2015 to 31st December 2015.

Methods: In 2015, a retrospective cohort study was undertaken and the data was analyzed by the Statistical Package for Social Science (SPSS) version 22.0. Independent sample T-test were used to compare the change in Hemoglobin (Hb), Platelets and TLC. A 95% confidence level and p-values less than 0.05 were obtained.

Results: Out of all the 24 albino rats obtained from the Animal House of the University of Lahore, the mean (±SD) change of Hemoglobin, Platelets and TLC from baseline to end of treatment was 13.35(±0.34) & 14.05(±0.05), 626.50±(230.70) & 27.75±(4.78) and 6.00±(081) & 2.00±(0.00) respectively, with and without *Berberis vulgaris* supplementation, with a p-value of < 0.0001.

Conclusion: It is recommended for a better outcome in case of the cyclophosphamide-induced hepatotoxicity.

Keywords: Anemia, Hepatotoxicity, *Berberis vulgaris* supplementation and change of hemoglobin, TLC.

INTRODUCTION

The hemoglobin concentration below the acceptable range is called Anemia[1]. Anemia is encountered as a very common complication in the patients suffering from cancer and undergoing therapy with chemotherapeutic agents. This feature of anemia is encountered in more than half of the cancer patients undergoing chemotherapy, and it may also encountered in as much as 90% of the patients[2]. Anemia may also result from some underlying disease, bleeding or poor nutrition. It is suggested in the preliminary studies, especially in head and neck cancers as well as several soft tissue cancers like hepatocellular cancer and renal cancers, that the survival and loco-regional control after radiation therapy[3,4,5].

A negative effect on quality of life (QOL) and overall performance status with cancer is seen in anemia that worsens symptoms such as fatigue, weakness, and dyspnea. It would be reasonable to take a proactive approach in identifying populations who need treatment for cancer-associated anemia (CAA) to provide timely management and to physical functioning, QOL, and prognosis in patients with cancer. An effective way to replenish the depleted hemoglobin level is transfusion but unfortunately, the side effects are very serious and may lead to increased mortality. It is observed in randomized clinical trials that erythropoiesis-stimulating agents (ESAs) produce significant increase in Hb level, decrease transfusion requirements, and improved QOL[6,7,8]. Such agents do not illicit any response in about half of the patients[6,7,8]. It had been seen in many studies that in the cases whose Hb level reached >12g/dl, ESAs shorten overall survival time, or time to tumor progression. The cases included in these studies belong to primary cancers of various organs like lung, liver, kidney, head, cervix, neck, breast and lymphoma[9,10,11]. The lack of response to erythropoietin stimulation in patients with cancer is partly attributed to the functional iron deficiency state, in which the high rate of erythropoiesis exceeds the delivery of usable iron, despite adequate iron stores[12,13].

In the treatment of anemia in patients with cancer, many randomized trials examined the role of iron in addition to ESAs. Reduction in ESA dose, improvement in ESA response, time to maximal response and QOL parameters (when measured), in favor of the combination over ESAs alone, is shown in many of these studies[14,15] .

The efficacy and feasibility of an herb root extract of Berberis vulgaris as a monotherapy in cases with liver cancer and liver toxicity induced by Cyclophosphamide, who have anemia and who are undergoing treatment with chemotherapy, is assessed in this pilot study. Therefore, the study aimed to answer the question whether *Berberis vulgaris* have a better improvement for anemia among rats suffering from Cyclophosphamide-induced Hepatotoxicity or not.

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METHODS AND MATERIALS

In the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, a retrospective cohort study was conducted from March to December 2015. Animals were obtained from the Animal House of UOL and put in cages in the groups of four after properly tagging them. As part of other clinical evaluation modalities, the hematology/blood chemistry, and other investigations are determined.

In the present study, twenty four (24) male albino rats were used, each weighing about 120-200 g. The Cyclophosphamide that was used in this study was a product of Pharmedic Laboratories (pvt, limited) and the roots of *Berberis vulgaris* plant ware used. The male albino rats were segregated in to six (6) group, each consisting of four (4) rats (n=4). The total span of the experiment was thirteen (13) days and the dissection of the animals was carried out on seventh (7^{th}) and thirteenth (13^{th}) day.

Berberis vulgaris roots were collected from Swat valley in northern areas of Pakistan, air dried at room temperature for 2 weeks and extract was prepared by crushing and dipping 100 gm of material in 500 ml of 70% methanol in water in a well capped glass jar. Jar was kept at room temperature, away from direct sunlight and manually swirled two times in a day for 5 minutes. After 2 weeks liquid was filtered and concentrated to dryness on rotary evaporator at 45℃.

The dose of *Berberis vulgaris* was prepared in distilled water and administered at 1000 mg/kg of the body weight of the rats. The Berberis vulgaris as given orally via gastric intubation to the rats. Cyclophosphamide was administered at the dose of 80 mg/kg of the body weight of the rats and it was injected in to the rats via intraperitoneal route. At the dissection, the blood samples were collected directly from the heart of each rat through heart puncture under the chloroform anesthesia. Blood samples were collected in the CBC vials containing EDTA and the collected blood samples were centrifuged within one hour of collection of samples. The serum extracted after the centrifugation was processed and analyzed for the estimation of further biochemical analysis. Dose Regimen of Cyclophosphamide and Berberis vulgaris:

1. Group I (control group), administered with normal diet and water and dissected on 13^{th} day.

2. Group II (Negative control group), the animals received 80mg/kg cyclophosphamide alone for 6 days intraperitoneally to induce toxicity and dissected on 7^{th} day.

3. Group III (Plant control group A), the animals received 1000 mg/Kg of Berberis extract alone orally for 6 days and dissected on 7^{th} day.

4. Group IV (Plant control group B), the animals received 1000 mg/Kg of Berberis extract alone orally for 6 days and dissected on 13^{th} day.

5. Group V (Combination group), the animals received both 80mg/kg cyclophosphamide i.p and 1000mg/kg B. vulgaris extract orally for 6 days and dissected on 7^{th} day.

6. Group VI (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 13^{th} day.

RESULTS

Statistical software package SPSS version 22.0 was used to analyze the collected data statistically. The data that was obtained during the study was entered in to SPSS 22.0. Moreover, the graphs and the tables shown were also generated using the SPSS 22.0 and Microsoft Excel 2013. To compare the individual and comparative changes in Hb, platelets and TLC, Independent sample T-test was used. A 95% confidence level and p-values less than 0.05 were used to determine statistically significant.

For the control group, the mean value of hemoglobin was calculated to be13.3±0.34. Massive effects of the drug was shown on the parameter in the negative control group where the mean value was intended to be 9.12±0.15, that was markedly decreased as compared to the mean value of the Hb levels in the control group. No effects of the plant extract on Hb levels were shown by the mean values of plant group A and plant group B, which were analyzed to be 13.95±0.64 and 13.85±1.14 respectively, being close to the mean value of the control group. Mean for the combination group was calculated to be 14.05±0.05. Whereas, the mean value of the prophylactic group was calculated to be 10.65±0.73. A marked decrease in the Hb levels were observed in the negative control group owing to the cyclophosphamide therapy. On the other hand, plant group A, plant group B and the prophylactic group showed a change towards normality.

In the control group the mean value of the platelet level was 626.50±230.70, which was observed to be decreased in the negative control group A, 73.75±0.95. The mean value of 27.75±4.78 in the combination group showed a significantly decreased level of PLTs. Both the plant group A and plant group B showed an elevated mean value of the platelet levels, 925.75±249.28 and 725.25±268.04 respectively. A very positive protective effect of plant extract was observed in the mean value of platelet levels that was calculated to be 7.75±1.89.

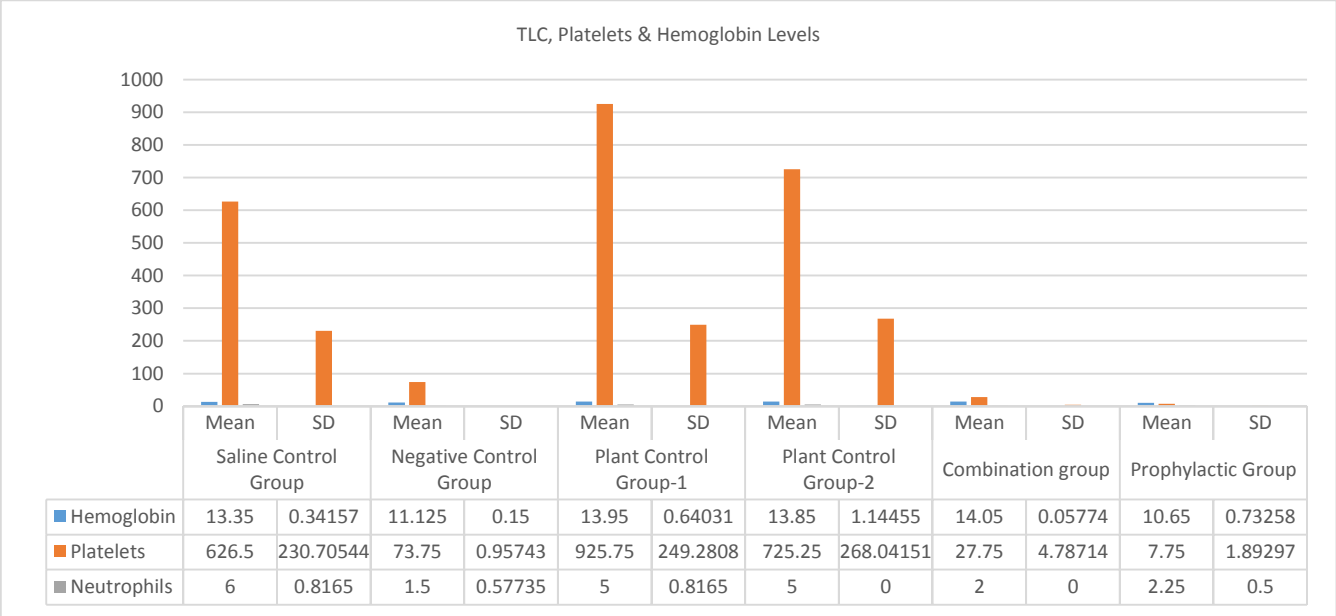
In the control group, the mean value of the neutrophils was observed as 6.00±0.80. Mean value of Negative control group recorded as 1.50± 0.57 (lower to mean of control group telling effect of drug). The mean values for the plant treated groups A and B were recorded to be 5.0±0.81 and 5.00±0.00 respectively, having closer values to the saline control group. The mean values of the prophylactic group and the combination group for the neutrophils was calculated to be 2.25±0.50 and 2.00±0.00 respectively. The last group which was pretreated with plant and extract had the mean value of 2.25±0.50, depicting marked difference from the toxicity groups and shifting towards the control group as a sign of protective effects of *B.*vulgaris.

In analysis of variance, for the values of Hb are calculated to be significant. Hb levels mean square values between groups and within groups were 9.37 and 0.40 that is considerably high to assume it significant and significance value is elevated to 0.001 from p value 0.05 as well.

Table1: Descriptive statistics (Mean±SD) of Total Leucocytes Count (103/mm^3), platelets (103/mL) and Hb (g/dL) levels in control group, groups suffering from cyclophosphamide induced toxicity, Berberis group (given alone for 6 and 13 days) and groups treated with Berberis vulgaris before and in combination with cyclophosphamide-induced toxicity.

Groups (Mean±SD)	Hemoglobin	Platelets	Neutrophils
Saline Treated Group	13.35±0.34	626.50±230.70	6.00±081
Negative Control Group	11.12±0.15	73.75±0.95	1.50±0.50
Plant Control Group-1	13.95±0.64	925.75±249.28	5.00±0.81
Plant Control Group-2	13.85±1.14	725.25±268.04	5.00±0.00
Combination Group	14.05±0.05	27.75±4.78	2.00±0.00
Prophylactic Group	10.65±0.73	7.75±1.89	2.25±0.50

Fig 1: Mean values (Mean ±SD) of Total Leucocytes Count (103/mm3), platelets (103/mL) and Hb (g/dL) levels in control group, groups suffering from cyclophosphamide induced toxicity, Berberis group (given alone for 6 and 13 days) and groups treated with *Berberis vulgaris* before and in combination with cyclophosphamide-induced toxicity.



	Saline Control Group		Negative Control Group		Plant Control Group-1		Plant Control Group-2		Combination group		Prophylactic Group	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hemoglobin	13.35	0.34157	11.125	0.15	13.95	0.64031	13.85	1.14455	14.05	0.05774	10.65	0.73258
Platelets	626.5	230.70544	73.75	0.95743	925.75	249.2808	725.25	268.04151	27.75	4.78714	7.75	1.89297
Neutrophils	6	0.8165	1.5	0.57735	5	0.8165	5	0	2	0	2.25	0.5

Table2: Analysis of Variance of Total Leucocytes Count (103/mm3), platelets (103/mL) and Hb (g/dL) levels in control group, groups suffering from cyclophosphamide induced toxicity, Berberis group (given alone for 6 and 13 days) and groups treated with *Berberis vulgaris* before and in combination with cyclophosphamide-induced toxicity.

ANOVA Table			Sum of Squares	df	Mean Square	F	Sig.
Hemoglobin * Groups	Between Groups	(Combined)	46.852	5	9.370	23.434	.000
	Within Groups		7.197	18	.400		
Platelets * Groups	Between Groups	(Combined)	3329371.208	5	665874.242	21.338	.000
	Within Groups		561718.750	18	31206.597		
Neutrophils * Groups	Between Groups	(Combined)	73.875	5	14.775	46.252	.000
	Within Groups		5.750	18	.319		

* = Significant as p<0.05

DISCUSSION

A nitrogen mustard alkylating agent, Cyclophosphamide, which imparts its antineoplastic function by forming the cross-linkages with DNA of the tumor is metabolized in liver to the active structure and eliminated via kidneys primarily. Owing to its role in detoxification of the xenobiotics and metabolic by-products, the liver is at risk to be damaged due to direct experience to noxious products. With a consequent reduction in their biosynthesis and altered membrane permeability permitting enzyme leakages into the serum, the elevation in the liver enzymes activities may be due to liver dysfunction[16]. Markedly decreased level of Hemoglobin, increased liver toxicity and mortality and decreased survival were the outcomes that were observed in this study due to the increased exposure to toxic metabolites of cyclophosphamide. When compared with normal control group rats, a marked increased levels of hemoglobin levels were observed. Berberine, when administered along with the Cyclophosphamide, resulted in the potential alleviation of the deranged serum levels of Hb. The Hb levels that were observed to be declined in the rats those were given cyclophosphamide, whereas, the Hb levels were significantly increased in the group concurrently

received berberine and CP (both). The remedial effects of herbs have been renowned from the many prehistoric civilizations to treat such toxicities. The potential of herbs and plant-based remedies have progressively been known for their preventive action and for the treatment of various human diseases and the plants are performing the role of major health care resources in all over the world. About a quarter of the drugs that are recommended these days are made from the extracts of plants or their effective component[17].

Berberis vulgaris (family *Berberidaceae*) is one of the significant medicinal plants that have been used for various therapeutic purposes. That is why, the aim of the current study is to report the effects of *Berberis vulgaris* root extract supplementation on variation of hemoglobin levels, platelet levels and total leukocyte count among rats suffering from cyclophosphamide-induced hepatotoxicity especially in albino rats. The current study was designed in a way that we could assess the comparison of the effects of *Berberis vulgaris* in pre-treatment and post-treatment, showing which would yield accelerated and elevated recovery. Quite a few studies that were undertaken recently had observed the possible role of antioxidants in protecting the liver and maintain the hemoglobin levels, platelet levels and TLC against the cyclophosphamide-induced toxicity. The protective role of various plants preparations having antioxidant properties had been studied to counter the toxicities resulted from cyclophosphamide exposures. 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[18]. Therefore, to overcome and counter the deranged Hb levels, platelets levels and TLC due to the toxicity rendered by the cyclophosphamide, the methanolic extract of *Berberis vulgaris* was employed.

CONCLUSION

A marked improvement of hemoglobin levels, platelet levels and TLC among the rats suffering from Cyclophosphamide induced hepatotoxicity was observed with the experimental *Berberis vulgaris* supplementation at 1000mg/kg body weight of the rats.

The methanolic root extract of *Berberis vulgaris* can play an important ameliorative role in the prophylactic treatment against the Cyclophosphamide - induced effects in blood (especially to counter the hemoglobin, platelet and TLC issues). However, the pre-treatment (prophylactic group) with *Berberis vulgaris* root extract showed significant results as compared to the combination group, in which the Cyclophosphamide and *Berberis vulgaris* were given simultaneously.

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