ORIGINAL ARTICLE

Insight into the Hypoglycemic Effects of Traditional Herb- *Berberis Vulgaris* Methanolic Root Extract Supplementation among rats in Treatment of Cyclophosphamide-Induced Hepatotoxicity

HAROON HABIB[1], YASIR ALI BHATTI[2], JHANGIR ZAIB[3], *MUZAMIL LIAQAT ALI[4], ALI IFTIKHAR[5], MUDASER HUSSAIN ABBASI[6]*

[1]Assistant Professor Biochemistry, Avicenna Medical College, Lahore
[2]Assistant Professor Biochemistry, Avicenna Medical College, Lahore
[3]Assistant Professor Medicine, Poonch Medical College, Rawlakot,
[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, Sarghoda.
Correspondence to Dr. Haroon Habib, Email: dr.haroonhabib@gmail.com

ABSTRACT

Background: In the current study, 24 healthy adult male albino rats each having a weight in between 120-200g were used, acquired from The University of Lahore, Animal House. The Berberis vulgaris was fed to the Cyclophosphamide-induced hepatotoxic rats, resulting in a serious complication of hypoglycemia. However, there is hardly any evidence shows any improvement in glycemic control due to supplementation by Berberis vulgaris methanolic root extract.

Aim: To accomplish a principal pharmacological screening contained in the aqueous extract of Berberis vulgaris and to discern the effects of hypoglycemia due to aqueous extracts on groups of rats rendered hepatotoxicity after injecting cyclophosphamide.

Place of study: Study was conducted in the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore.

Duration of Study: 01 year from 01 January 2015 - 31 December 2015

Methods: A retrospective cohort study was conducted from January 2015 to December 2015. Data were formulated into Microsoft Excel sheets Version 2013 and for analysis transferred to Statistical Package for Social Science (SPSS) version 22.0. A change in Hb was compared by using Independent T-test. Lastly, to determine significance statistically, the p-values less than 0.05 and a 95% confidence level were used.

Results: From a total of 24 albino rats, all were from Animal House of The University of Lahore. The mean change (±SD) of Hb from starting point to end of treatment was 13.35(±0.34) and 14.05(±0.05) g/dl with and without Berberis vulgaris supplementation respectively, with a p-value of < 0.0001 that is clearly associated with the variation of Hb.

Conclusion: Due to the positive effect of Berberis vulgaris root extract supplementation on the change of Hb, it can be recommended for an improved outcome in case of cyclophosphamide-induced hepatotoxicity.

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

INTRODUCTION

Berberis vulgaris Linn also known as Barberry, belongs to the Berberidaceae family. Berberis is the genus of spiny deciduous evergreen shrubs, comprising flowers and yellow wood, and have almost 190 species. Traditionally the extracts of many Berberidaceae are medicinally utilized as hepatic tonic and to treat various chronic inflammations[1]. According to some authors, these extracts possess a remarkable activity against viruses, fungi, helminths, protozoa and bacteria such as chlamydia[2]. As far as chemical components and various properties are concerned, the research shows that the principle activity is produced as a result of their alkaloid components, possessing isoquinolinic nucleus, for instance oxycanthine, berberine, palmatine and berbamine[2]. Berberine has also been found to be associated with hypotensive, anti-inflammatory, anti-arrhythmic, antimicrobial, febrifugal and immune-stimulating characteristics, as well as, in prolonging the duration of action potential in Purkinje fibers. Many on-going studies suggest a possible anti-tumor activity role of berberine[1-5]. Liver being a vital organ is responsible for secretion, metabolism and waste products removal. In addition, it plays an important role in maintenance, implementation and body homeostasis regulation. Many biochemical growth pathways, defense against diseases, nutritional supply, reproductive processes and production of energy mechanisms are all dependent on liver. Many researchers believe in relationship of liver with the production of all illnesses in comparison to the deficiency of other body organs to function properly [6]. One of such pivotal roles of liver is to aid the metabolism involved with digestion of various foods, most of the medicines and alcohol. The use of medicines can result in liver injury ranging from mild abnormality in its function, evidenced by raised serum aminotransferase levels to extensive damage such as necrosis of hepatocellular tissue or hepatotoxicity due to intraperitoneal cholestasis[7].

Diabetes mellitus (hyperglycemia) is a major disorder in humans involving multiple manifestations clinically. It is a

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syndrome characterized by increased blood glucose levels in consequence to relative or absolute insulin deficiency. In line with the World Health Organization projections, the diabetes population is expected to be 300 million or more by the year 2025 [8-9]. At present, available treatment modalities for diabetes mellitus affected cases contain insulin and multiple oral hypoglycemic drugs, either utilized as monotherapy or in combination to attain improved glycemic control. The information about anti-diabetic potential and ethnobotanical details of quite a few plants are acknowledged at present [10-13] however there is insignificant information related to plants that have hypoglycemic properties. The hypoglycemic activity could be as a result of the presence of polyphenols, saponins and alkaloids[14].

This pilot study evaluated the usefulness and probability of a Berberis vulgaris herb root extract as a mono therapeutic intervention in chemotherapy induced liver toxicity and anemia by the use of Cyclophosphamide in patients having liver cancer. Consequently, the study focused to answer the query whether *Berberis vulgaris* have a better progress for elevated levels of glucose amongst rats suffering from hepatotoxicity induced by Cyclophosphamide or not.

METHODS AND MATERIALS

An organization centered retrospective cohort study was piloted from March 2015 to December 2015 in the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan. Experimental animals were confined and acquired from the Animal House of UOL. The chemistry of blood / hematology, and other investigational parameters are measured as part of other relevant clinical assessment modalities. Nutritional assessment, such as BMI, and weight are measured at the time of admission, weekly and then at dissection. Besides Cyclophosphamide induction, the BV supplementation to these rats is also given.

Sample size and sampling procedure: In a current study, with an average weight of 120–200g, 24 healthy adult albino male rats were used. Roots of *Berberis vulgaris* and Cyclophosphamide were used was where a later sourced from Pharmedic Laboratories. In total, six groups were designed each comprising of 04 rats (n = 4). A total duration for experimentation was of 13 days where the dissection was done only at 7th and 13th day. Dosing time was used to be at 02:00pm. The doses of Cyclophosphamide and Berberis Vulgaris (80 and 1000 mg/kg. Body weight) respectively, were prepared in distilled water. Intraperitoneal route was used for injecting Cyclophosphamide whereas, Berberis vulgaris was given by oral route with the aid of gastric intubation. Using chloroform anesthesia, the heart puncture of each rat was performed to collect blood from the heart directly. The collected blood sample was centrifuged to isolate serum within an hour of collection of whole blood in CBC vials having EDTA. The samples containing serum were processed and analyzed for determining additional biochemical analysis.

The collection of *Berberis vulgaris* roots were from the valley of Swat in Pakistan northern region. The preparation of extract involved air drying at room temperature for 14 days followed by the crushing and dipping 100 gm of material in 500 ml of 70% methanol in water in a fit capped glass jar. The jar away from direct sunlight was kept at room temperature and manually churned twice a day for at least 5 minutes. The liquid was finally dried using a rotary evaporator at 45℃ after undergoing filtration and concentration processes.

Dose Regimen of Cyclophosphamide and Berberis vulgaris:

1. **Group I (control group)**, administered with normal diet and water and dissected on 13th day.
2. **Group II (Negative control group)**, the animals received 80mg/kg cyclophosphamide alone for 6 days intraperitoneally (i.p) to induce toxicity and dissected on 7th 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 7th 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 13th 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 7th 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 13th day.

RESULTS

The statistical analysis was conducted on the data using statistical software package SPSS version 22.0 for windows. 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. Independent sample T-test were used to compare the change in Hb. A 95% confidence level and p-values less than 0.05 were used determine statistically significant

Table 1: Descriptive statistics (Mean±S.D) of Blood sugar (mg/dl) levels at day 1,7 and 14 and Body weight (grams) at day 1, 7 and 14 in control group, negative control group, plant control groups and groups suffering from cyclophosphamide induced toxicity.

Mean value of Blood sugars (mg/dl) were calculated to be 100.50±6.45 for control group. In negative control group mean value was intended to be 94.75±8.26. Mean values for plant control group-1 and plant control group-2 were analyzed as 101.25±16.74 and 103.75±21.26 respectively that were close to control group. Mean for the combination group was calculated to be 96.50±8.69. The mean value of Prophylactic group was computed as 98.25±20.35. All these groups were showing change towards normality or normal values at day 1 and considered as baseline. While at day 7 and day 14, saline treated group showed mean values of 101.75±11.92 and 90.25±15.65. In negative control group the mean values were 158.50±16.78 and 168.75±17.05. While the plant control group-1 and plant control group-2 showed the mean values as 61.00±5.47 & 55.75±5.12 and 61.50±7.41 &

60.25±6.70 respectively. The combination group was calculated as 106.50±16.66 and 104.00±13.58 at day 7 and 14 respectively while the prophylactic group showed the mean values of 64.50±4.20 and 72.50±6.95 at day 7 and 15 respectively

In Analysis of variance, for the values of Blood sugar were calculated to be significant. Blood sugar levels mean square values between groups and within groups were 0.000 that is considerably high to assume it significant and significance value is elevated to 0.001 from p value 0.05 as well.

Groups	Blood Sugar (mg/dL) Day 1	Blood Sugar (mg/dL) Day 7	Blood Sugar (mg/dL) Day 13	Body Weight Day 1 (g)	Body Weight Day 7 (g)	Body Weight Day 13 (g)
Saline Control Group	100.50±6.45	101.75±11.92	90.25±15.65	128.00±6.73	146.50±8.54	163.50±9.29
Negative Control Group	94.75±8.26	158.50±16.78	168.75±17.05	164.00±8.16	129.00±6.00	-
Plant Control Group-1	101.25±16.74	61.00±5.47	55.75±5.12	164.00±5.65	177.00±8.24	-
Plant Control Group-2	103.75±21.26	61.50±7.41	60.25±6.70	146.50±9.71	163.00±7.39	175.00±11.83
Combination group	96.50±8.69	106.50±16.66	104.00±13.58	163.00±7.74	135.50±9.98	120.00±7.11
Prophylactic Group	98.25±20.35	64.50±4.20	72.50±6.95	162.00±9.38	176.50±5.74	155.00±10.52

Fig 1: Mean values (Mean ±SD) of Blood sugar (mg/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.

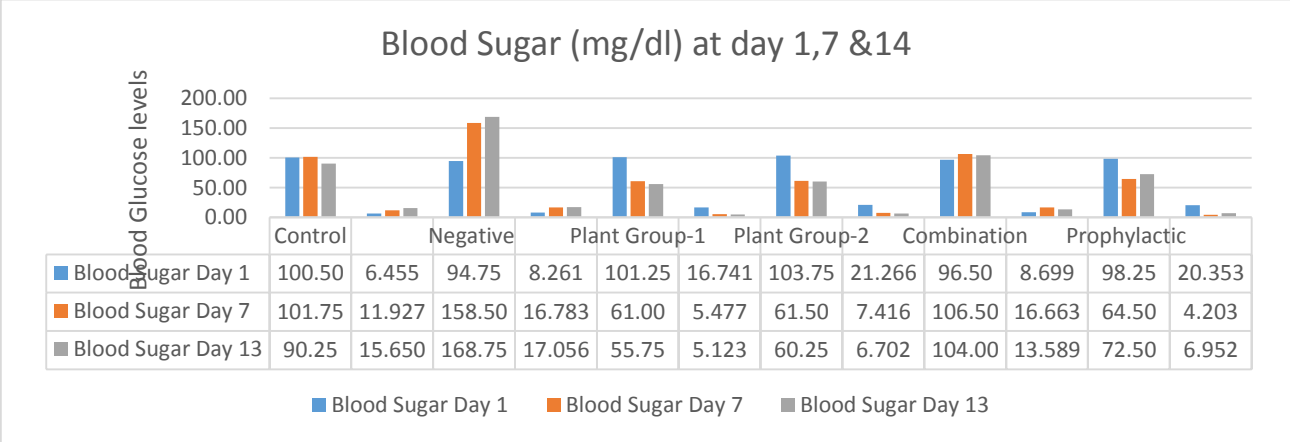

Table 2: Analysis of Variance of blood sugar (mg/dL) levels and body weight (grams) in control group, groups suffering from cyclophosphamide induced toxicity, Berberis group (given alone for 6 and 13 days) and groups treated with Berberis vulgaris before and in combination with cyclophosphamide-induced toxicity.

Groups			Sum of Squares	df	Mean Square	F	Sig.
Blood Sugar Day 1	Between Groups	(Combined)	218.333	5	43.667	.197	.960
	Within Groups		3997.000	18	222.056		
Blood Sugar Day 7	Between Groups	(Combined)	29498.208	5	5899.642	44.013	.000
	Within Groups		2412.750	18	134.042		
Blood Sugar Day 13	Between Groups	(Combined)	34959.833	5	6991.967	49.943	.000
	Within Groups		2520.000	18	140.000		
Body Weight Day 1	Between Groups	(Combined)	4300.833	5	860.167	13.359	.000
	Within Groups		1159.000	18	64.389		
Body Weight Day 7	Between Groups	(Combined)	8550.833	5	1710.167	28.164	.000
	Within Groups		1093.000	18	60.722		
Body Weight Day 13	Between Groups	(Combined)	6746.750	3	2248.917	23.205	.000
	Within Groups		1163.000	12	96.917		

* = Significant as p<0.05

DISCUSSION

Cyclophosphamide is a nitrogen mustard alkylating agent that exhibits its anticancer role by the formation of cross-linkages with the tumor DNA. It is metabolized mainly in liver to active configuration and eliminated largely via kidneys. The liver is largely at risk to a damage induced by direct exposure to toxic products on account of its role in detoxification of metabolic byproducts and xenobiotics. In the past, several medicinal plants or their extracts in old-fashioned folk medicines were used to treat a state of hyperglycemia [15]. The present study demonstrates the evaluation of reduced serum glucose levels due to the effect of methanolic root extract of *B.vulgaris.* At day 7, the extract administered once, significantly reduced the blood glucose levels and that extract showed an enhanced activity in an aqueous form. These interpretations may favor the nature of biologically active compound. Recent

studies also show that saponins as well as plant metabolites are well known for their antihyperglycemic activity. This enhanced activity could be due to existence of alkaloids, flavonoids, tannins, saponins and steroids constituents. Diverse mechanisms to lower blood glucose levels with the aid of plant extracts already exist. It is assumed on the basis of results that the root extracts could be helpful tools for stimulating insulin release in addition to the observed restoration of metabolic activities. Likewise, the results of the present study suggest that the extracts possessing active phytochemicals postulates with cell-protective functions either on the insulin-protective properties or the pancreatic beta cells. Likewise, a variety of other plants have also been revealed which enhance hypoglycemic activity by stimulating insulin release[18].

In the present study, the pre-treatment was compared with post-treatment in order to find that which treatment (either pre or post treatment) enhanced more recovery. A recent research suggests the potential role of antioxidants in favor of liver defense and to maintain the glucose levels against cyclophosphamide-induced hyperglycemia. A number of plant preparations with numerous antioxidant potentials were also observed as protective mediators against toxicities caused due to exposure of cyclophosphamide. Recent research data also revealed that the *Berberis vulgaris* have quite a lot of therapeutic components with striking antioxidant effects for example berberine. According to various recent studies, the methanolic root extract of *Berberis vulgaris* owns remarkable antioxidant properties[17].

CONCLUSION

The aqueous extract of root bark of *Berberis vulgaris* showed remarkable antihyperglycemic effects in cyclophosphamide-induced hepatotoxic rats. The results propose that the hypoglycemic effect was due to the presence of saponins which may have stimulating effect on the remnant beta cells. Conversely, additional experimentation is required to interpret the more precise mechanism of action. Improvement in other parameters were showed by these extracts, so may be very valuable in the treatment of diabetes mellitus.

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