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# Evaluation of hepato-protective activity of *B. lycium* methenolic crude extracts collected from Distric Sherani, Balochistan

**Sami Ullah Sherani[1], Javied Iqbal[1], Amanullah Khan[2], Muhammad Ali Khan*[1], Nadeem Rashid[1], Mohammad Rahim Niazi[1], Zia Ud Din[1], Mohamad Kamran Taj[1]**

[1]*University of Balochistan, Quetta, Pakistan*

[2]*Health Department Balochistan, Quetta, Pakistan*

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**Key words:** *Berberis lycium*, *Sherani,* Hepatoprotective activity, Balochistan, LFT, PP.

**Abstract**

*Berberis lycium*, which is commonly called as Indian barberry (English) and *Kashmal* or *Ishkeen* in Urdu, *is* a spiky plant which is the member of the genus Berberis of family Berberidaceae. *B. lycium* also includes anti-hepatotoxicity effect, when was mixed with *G. aparine* and *P. integerrima* and was tested in rats that were treated with carbon tetra chloride; the results revealed that the combination of these three medicinal plants encompasses anti hepatotoxicity effects. *B. lycium* (root) sample was collected from the hill of village of *Ahmadedergah* near to *Tahkhta Suleiman* District *Sherani* Balochistan, Pakistan. The collected samples were identified by Pharmacognosy, Department faculty of Pharmacy University of Baluchistan, Quetta. Adult healthy rabbits (male) having weights approximately 950g-1300g, were kept in animals house at CASVAB, UoB. Sample serum, within 3 hours after collection , was analyzed for certain biochemical parameters (Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Gamma glutamyl transpeptidase (γ-GT), Aspartate aminotransferase (AST), and total Proteins like Bilirubin, Albumin and Globulin) through automatic analyzer (Merck) at 37°C through standard reagent kits. The obtained values for LFT in the current portion of controlled group were, 0.76± 0.060, 0.032±0.008(mg/dL), 4.2±0.802, 93.8±1.500, 2.28±0.107 and 273±2.818(U/L) for TB, DB, ALT, AKP, GGT, SGOT and SGPT (AST) levels. Whereas, the group treated with $CCl_4$ these parameters were, 0.86±0.075, 0.064+0.006(mg/dL), 4.6±0.680, 2.44±0.150, 90.4±3.467 and 218.4±1.439(U/L) level. While, the group treated with $CCl_4$ and *B. lycium* 500mg, the above parameters were calculated as 0.92±0.086, 0.442±0.228(mg/dL), 4.4±0.601, 93.2±1.244, 2.62±0.097 and 221.6±1.540(U/L), respectively.

***Corresponding Author:** Muhammad Ali Khan ✉ drkhanpishin@yahoo.com

## Introduction

In Pakistan, many native plants are utilized in herbal medicine to treat diseases and injuries. Such plants often shows a broad spectrum of biologic and pharmacologic activities, for example, they are able to reduce inflammation, having antibacterial and antifungal properties Cowan MM. "(1999). The root, bark, seed and fruit extracts of the medicinal plants are used in syrups and infusions, traditionally (Imtiaz and Manzoor, 2003).

*Berberis lycium*, which is commonly called as Indian barberry (English) and *Kashmal* or *Ishkeen* in Urdu, *is* a spiky plant which is the member of the genus Berberis of family Berberidaceae. (Sabir *et al*., 2013). *It* is found in the moderate and semitropical Asian, European and American divisions (Jabeen *et al*., 2015). In Pakistan it is extensively distributed in Baluchistan, KPK and Punjab provinces, especially in northerly regions, Swat and Azad Kashmir at elevation of 900 to 2900m (Dhar *et al.,* 2012)

*B. lycium* is a vertical flowering bush plant that increases to a length of 3-4 meters, having a solid timber stem and is enclosed in a slight fragile bark. The branches of *B. lycium* are light white to grayish and have thorns alternatively fixed on them (Ahmad and Sharif, 2009). Root bark could be approximately 3 mm solid, from the outside fractured and inside smooth (Chauhan, N. S. (1999) *B. lycium* is extensively utilized for the treatment UTI, swelling of spleen, stomach and intestinal ulcer and liver diseases (Irshad *et al*., 2013). Local population utilizes the powdered form of dried root bark after combining with dissolved animal fat for bone fractures as a bandage. Shoots of the plant are employed for the abdomen pain, jaundice and loose bowels (Beers, 2012). The bark of the plant has wound healing activity (Asif, *et al.,* 2007).

*B. lycium* also includes anti-hepatotoxicity effect, when was mixed with *G. aparine* and *P. integerrima* and was tested in rats that were treated with carbon tetra chloride; the results revealed that the combination of these three medicinal plants encompasses anti hepatotoxicity effects. (Atawodi *et al.*, 2011). To estimate Hepatoprotective effect of *B. lycium*, crude powder and Methanolic extract of plant were used. $CCl_4$ was given to the rabbits to induce hepatotoxicity. Results showed that plant considerably decreased the elevated levels of serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase and alkaline phosphatase enzymes in hepatotoxic rabbits (Malik *et al.,* 2011).
The present study was therefore designed to evaluate the in vitro Hepatoprotective effect of *B. lycium*.

**Fig. 1 .** *B. Lycium.*

## Materials and methods

*Sample collection*

*Berberis Lycium* (root) sample was collected from the hill of village of *Ahmadedergah* near to *Tahkhta Suleiman* District *Sherani* Balochistan, Pakistan. The collected samples were identified by Pharmacognosy, Department faculty of Pharmacy University of Baluchistan, Quetta under the specimens voucher (No. S-243).

**Fig. 1.** Ahmadedergah near to Tahkhta Suleiman District Sherani.

*Sample preparation*

After the collection of the plant roots following procedure was adapted;-

1. The fresh plant of B. lycium roots were exposed to washing and chopping,

2. then desired materials were left for 60 minutes (Lust, 2014).
3. After that the herb was cut into small pieces.
4. Dehydrated is shed at 25oC for 30 days.
5. The dried fragments were milled into fine powder.
6. Macerated in methanol at room temperature, occasional shaking as well as stirring done on alternate day for fifteen days followed filtration.
7. The extracts obtained were concentrated via Heidolph rotary serial number 519-00000-003, made of (Germany) evaporation at (45°C).
8. Semi-solid (CMEs) was obtained, kept at 4°C until screened for further processing. (Kujur *et al.,* 2010).

*Application*

Adult healthy rabbits (male) having weights approximately 950g-1300g, were kept in animals house at CASVAB, UoB. The selected animals were kept in metal cage and feed with clean water and food throughout the study period under standard protocol of relative humidity, temperature (22±1°C) and 12hrs light/dark cycles and adapted for 1-week earlier the test in the research Laboratory of CASVAB. Before conducting the research, the procedure for research was approved by the Animal Ethics Committee (AEEC) of university of Balochistan, Quetta.

Silimyarin (DTL) liquid paraffin and Media of analytical grade were used in in the experiments. (Rauf, *et al.*, 2015)

The dose were based on the body weight (mg/kg) and calculated through following formula

$$\frac{\text{Animal weight (kg) x dosage (mg/kg)}}{\text{Concentration of drug (mg/ml)}} = \text{Drug volume in ml or cc}$$

The Methanolic crude extracts (MCE) were dissolved and diluted with distilled water. Controlled animals were treated with an equal volume of saline solution (Nair & Jacob, 2016).

The oral route was used for plant extract while for the standard and control oral and intraperitoneal (i.p) and subcutaneously route were used (Ribeiro. 2000).

1. Rabbits were adapted for seven days before experiment and housed in standard temperature humidity and light conditions with 12 hour light/ darkness cycle (Good Laboratory) were used. (Aniagu *et al.,* 2005).
2. Rabbits weighing 1000-1400 gm in the control group (group 1), NaCl 0.9% (1ml / Kg)
3. The treated group was given $CCl_4$ (Carbon tetrachloride) (Group II).
4. Plant extract (MCE) dose 500mg/kg/day was given to Group III
5. Standard drug (Silimyarin) of dose 100 mg/kg/day administered to Group IV. (15 days)
6. On the sixteenth day the Groups 2.3,4 were administered $CCl_4$, with 1:1, 1.5ml/Kg liquid paraffin afterward 30 min of the 2nd dosage of vehicle. (Kumar, *et al.*, 2011)
7. Group IV was treated with Silimyarin 100µg / kg bw weight group along with $CCl_4$.
8. Group III were treated with MCE with the concentration of 500 mg/kg body weight, respectively with $CCl_4$ (Sureshkumar & Mishra, 2008).
9. After 36 hours of last dose, 5cc blood were taken in Eppendorf via cardiac puncture technique to obtain the serum. The samples were kept in centrifuge machine up to 6000 rpm for ten minutes (Feroz *et al.,* 2011b; Etang *et al.,* 2013).
10. Sample serum, within 3 hours after collection, was analyzed for certain biochemical parameters Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Gamma glutamyl transpeptidase (γ-GT), Aspartate aminotransferase (AST), and total Proteins like Bilirubin, Albumin and Globulin) through automatic analyzer (Merck) at 37°C through standard reagent kits (Muhammed & Ahmad, 2014).

## Results

*Liver function Test (LFT)*

The obtained values for LFT in the current portion of controlled group were, 0.46± 0.060, 0.22±0.008 (mg/dL), 80.8±1.500, 2.28±0.107, 260± 2.818 and 3± 0.802 (U/L) for TB, DB, ALT, AKP, GGT, SGOT and SGPT (AST) levels. Whereas, the group treated with $CCl_4$ these parameters were, 0.86±0.075, 0.064+0.006 (mg/dL), 4.9±0.680, 2.44±0.150, 99.4±3.467 and 288.4±1.439 (U/L) level. While, the group treated with $CCl_4$ and *B. lycium* 500mg, the above parameters were calculated as 0.92±0.086, 0.442±0.228 (mg/dL), 4.0±0.601, 90.2±1.244,

2.62±0.097 and 270.6±1.540 (U/L), respectively. Silimyarin, was used as a standard drug treated with $CCl_4$ and the values for TB, DB, ALT, AKP, GGT, SGOT and SGPT were, 0.7±0.0838, 0.28±0.008 (mg/dL) 3.4±0.680, 86.8±4.544, 2.18±0.0802 and 210.6±2.587, (U/L), respectively.

**Table 1.** Hepatoprotective activity, effects of *B. lycium* in liver function test.

| Test | TB (mg/dL) | DB (mg/dL) | SGPT (ALT) (U/L) | Gama GT | AKP (U/L) | SGOT (AST) (U/L) |
|---|---|---|---|---|---|---|
| control (Normal Saline) | 0.46± 0.060 | 0.22±0.008 | 3± 0.802 | 2.28±0.107 | 80.8±1.500 | 260± 2.818 |
| $CCl_4$ treated | 0.86±0.075 | 0.064± 0.006 | 4.9±0.680 | 2.44±0.150 | 99.4±3.467 | 288.4± 1.439 |
| $CCl_4$+Extracts 500m/kg | 0.92± 0.086 | 0.442± 0.228 | 4.0±0.601 | 2.62±0.097 | 90.2±1.244 | 270.6± 1.540 |
| Stand+$CC_{l4}$ | 0.7± 0.0838 | 0.28± 0.008 | 3.4±0.680 | 2.18±0.0802 | 86.8±4.544 | 210.6± 2.58 |

TB= Total bilirubin, DB = direct bilirubin, SGOT= serum glutamic-oxaloacetic transaminase, ALT= Alanine aminotransferase , γ-GT = Gamma glutamyl transpeptidase, AKP =Alkaline phosphatase, AST = Aspartate aminotransferase, SGPT= serum glutamic-pyruvic transaminase

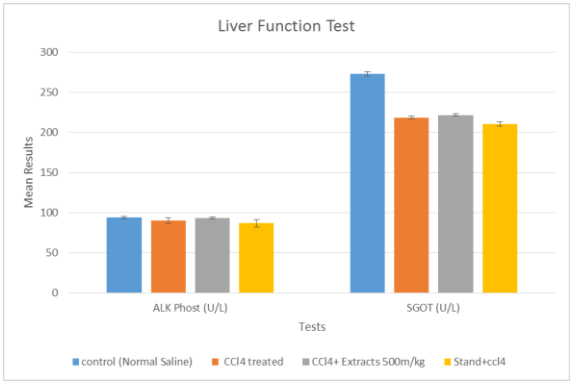


**Fig. 1.** Hepatoprotective activity, effects of *B lycium* in liver function test.

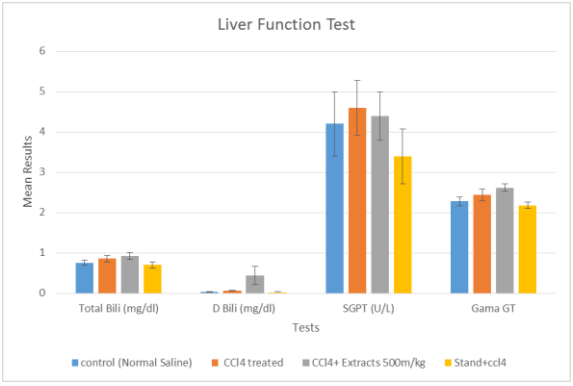


**Fig. 2.** Hepatoprotective activity, effects of *B. lycium* in LFT.

*Protein Profile*

In controlled group the values for Total Proteins, Albumin and Globulin, were 6.18±0.097, 3.38±0.132 and 3.42±0.165(g/dL), while, the values for the same parameters in $CCl_4$ treated group were, 6.54±0116, 5.24±012 and 7.17±0116(g/dL) respectively. Whereas, the same parameters were calculated in the group treated with *B. Lycium* 500mg/kg and $CCl_4$, the resulted values were 6.4±0.164, 3.86±0.081and 2.68±0.102(g/dL), respectively. Whereas, Silimyarin, was used as a standard drug treated with $CCl_4$ and the values for TP, Al and GB were observed as 6.36±0.133, 3.64±0.211 and 3.64±0.211(mg/dL), respectively.

**Table 2.** Hepatoprotective activity, effects of *B. lycium* on protein profile.

| | TP (mg/dL) | Al (mg/dl) | GB (mg/dL) |
|---|---|---|---|
| Controlled group | 6.18±0.097 | 3.38±0.132 | 3.42±0.165 |
| $CCl_4$ treated | 6.54±0116 | 5.24±012 | 7.17±0116 |
| $CCl_4$±Extracts 500m/kg | 6.4±0.164 | 3.86±0.081 | 2.68±0.102 |
| Stand+$CCl_4$ | 6.36±0.133 | 3.64±0.211 | 3.64±0.211 |

Values are the mean number of activities ± standard errors means, (n=5), ($P$<0.05)

PP= Protein profile, TP = total protein, Al = Albumin, GB= Globulin

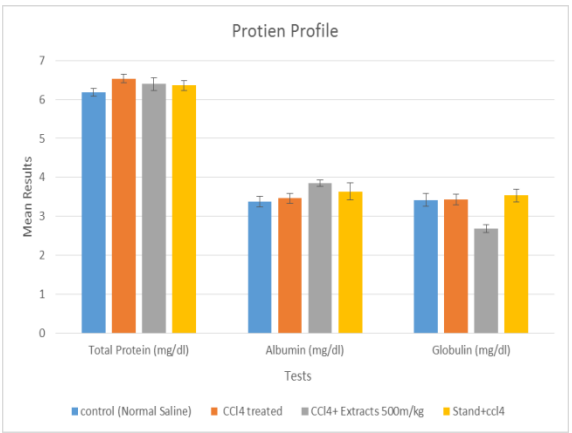


**Fig. 3.** Hepatoprotective activity, effects of *B. lycium* on protein profile.

## Discussion

Liver is the main organ of metabolism and energy production. It regulates systemic lipid homeostasis, which is involved in the redistribution of lipoproteins, triacylglycerol for storage and utilization by peripheral tissues (Lee *et al.*, 2003). Several plant extracts have been examined for use in a wide variety of liver disorders, *G. pentaphylla* protects membrane integrity in mice hepatocytes (Nayak *et al.*, 2011). *S. marianum* is a chemo-preventive agent that shows antitumor activity against human tumors in rodents (Agarwal *et al.*, 2006). Similarly, several cases of hepatotoxic side effects of green tea have been reported in rats (Rohde *et al.*, 2011).

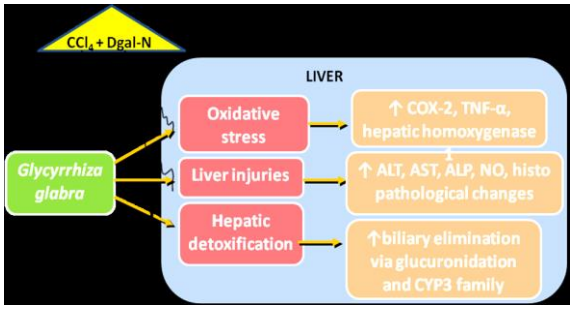


**Fig. 4.** Hepatoprotective activity of *G. glabra* due tri glycerol retinoic acid and liquorish by inhibiting the liver injuries and inflammation via controlling the oxidative stress parameters and increasing the hepatic detoxification via increasing the cytochrome phase I and glucuronidation phase II metabolism which become affected by Hepoto toxins carbon tetrachloride and D-galactosamine N (Razzuq *et al.*, 2012).

During the present study all the treated groups of the tested animals (rabbits) were given $CCl_4$ according to the recommended doses and evaluated serum TB, DB, Alanine aminotransferase (ALT), Alkaline phosphatase (ALP)., Gamma glut-amyl transferees (GGT), SGOT and Aspartate aminotransferase (AST or SGPT) by Liver function test (LFT), furthermore, total protein (TP), Albumin (Al), Globulin (Gb) were calculated through Protein Profile test (PFT) (Amin *et al.*, 2005).

It is revealed from the results of the this study that the Metholanic Crude Extracts (MCE) of *B. lyceum* at the dose of 500mg/kg body weight decreased serum TB, DB, ALT, AKP, GGT, SGOT and SGPT (AST) and TP, Al and Gb in the tested animals (rabbits) (Yang, *et al.*, 2006). Higher activities of these enzymes in serum have been found in response to oxidative stress induced $CCl_4$ (Yang, *et al.*, 2003). This was also evidenced from the downturn in levels of marker enzymes of test groups compared with the toxic control group and pure drug. The improvement in the enzyme activity was due to sustained and targeted action of Silimyarin and MCE on the hepatocytes due to their pronounced antioxidant effect may have diminished the release of SGOT, SGPT and ALP enzymes from the liver cells, thereby eliciting Hepatoprotective activity.

The findings of the present study also showed antioxidant properties, which are comparable to some previous reported studies of other plant extracts, having Hepatoprotective activity (Myagmar, *et al.*, 2004), *B. lyceum* showed strong free radical scavenging activity when extracted by methanol (Rong, *et al.*, 2007). The results of this study showed that after administration of MCE the activities of the serum marker enzymes were returned to near to the standard drug (Silimyarin) (Kim, *et al.*, 2008).

## Conclusions

Metholanic Crude Extracts of root of *B. lycium* showed remarkable Hepatoprotective activity in the experimental rabbits and the results were almost near to those of the Silimyarin drug, which was used as a standard drug during the study. Furthermore, the hepatotoxicity due to CCl4 can be reduced up to safe level through the usage of this plant extract.

These results have showed some light on the clinical therapeutic potential of these MCEof *B. lyceum* against hepatotoxic agents. More studies should be performed similar to this research in order to specify the main phyto compounds present in this plant which may be responsible for Hepatoprotective activity.

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