

Hepato-protective potential of aqueous extract of *Berberis lycium* Royle root bark extract in alloxan induced diabetic Swiss albino mice

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SUMMARY

Alloxan exposure damages the pancreas's beta cells and causes liver damage. Herbal remedies derived from plants are important in treating drug-induced liver damage. This study aimed to investigate whether pre-treatment with an aqueous extract of *Berberis lycium* Royle root bark could reduce alloxan-induced liver damage in Swiss albino mice. Various parameters, including protein, MDA, LDH, catalase, GSH, bilirubin, ASAT, ALP, and ALAT, were assessed. The mice were divided into four groups, and diabetes was induced using alloxan. The hepatoprotective activity of the BLR extract was then evaluated. The addition of alloxan increased the plasma levels of ALAT, ASAT, ALP, and LDH. However, pre-treatment with BLR extract significantly reduced these enzyme activities induced by alloxan. Alloxan induction caused an increase in bilirubin levels and a decrease in total protein content, which were restored by the BLR extract. Alloxan also led to an elevation in MDA levels and a reduction in GSH and catalase levels, which were reversed by the BLR extract. Overall, pre-treatment with BLR extract prevented liver damage induced by alloxan in mice, demonstrating its protective effect against liver damage.

Keywords: Alloxan, Hepatotoxicity, *Berberis lycium* Royle root bark extract, Swiss albino mice

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INTRODUCTION

The liver is a vital organ responsible for detoxifying and metabolizing harmful substances that enter the body. However, this process can sometimes result in liver damage and potentially life-threatening diseases (Ntamo et al., 2021). The liver is the primary target for most toxicity concerns (Ramappa and Aithal, 2013). Hepatotoxicants can cause oxidative stress to liver cells (Singh et al., 2016). Liver disorders caused by chemicals are primarily due to the production of free radicals, impaired antioxidant enzyme activity, and lipid peroxidation (Ighodaro and Akinloye, 2018). To treat liver diseases, antioxidant activity may be enhanced or free radical production may be reduced (Al-Dbass, 2012). There are various synthetic and natural therapies available for liver diseases (Hong et al., 2015), but herbal remedies derived

from plants are particularly important for treating drug-induced liver damage (Xiong and Guan, 2017). Numerous plants are used in the treatment of liver diseases.

The liver, which is the most crucial organ in the human body, plays a vital role in numerous important processes. One of these roles is regulating the glucose level in the blood (Kazankov et al., 2019). In the case of non-alcoholic fatty liver disease, a condition related to diabetes, the liver accumulates excessive fat. Diabetes is the major factor responsible for the development of this disease (Zawdie et al., 2018).

B. lycium Royle has been employed to treat various diseases and possesses diaphoretic, expectorant, diuretic, and febrifuge properties (Shabbir et al., 2016; Mughal et al., 2020; Mughal et al., 2022). Different parts of *B. lycium* Royle have been utilized to produce traditional medicines (Ahmed et al., 2004). Modern medicines derived from traditional medicine systems have successfully treated numerous diseases. Several reports have suggested that plant constituents exhibit exceptional properties by targeting newly discovered drug targets. Recent studies have shown that the use of *B. lycium* Royle lowers blood glucose levels, acting similarly to insulin. *B. lycium* Royle comprises an alkaloid called Berberine, which is renowned for its anti-inflammatory effects. Local experts have also used *B. lycium* Royle for wound treatment. The ancient knowledge of medicines continues to guide the development of current medicines and novel drug targets for the treatment of various conditions (Aggarwal et al., 2006; Mughal et al., 2020; Mughal et al., 2022). The objective of this research is to determine the hepato-protective impact of root and bark extract of *Berberis lycium* Royle in mice. The main aim is to identify an alternative to the existing medicine currently available in the market.

MATERIALS AND METHODS

Collection of medicinal plant

The *Berberis lycium* Royle plant was collected in Chinari, District Jhelum Valley, Azad Kashmir, Pakistan. It was identified by a botanist from the University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. To remove any dust, the plant was washed under running tap water. The dried plant pieces were then ground into a fine powder.

Preparation of plant extract

The water extract of *Berberis lyceum* Royle was prepared by following the methodology described in Mughal et al., 2020b and Mughal et al., 2022. Ten grams of shade-dried root bark were boiled in 100 milliliters of distilled water. After cooling, the extract was filtered through filter paper and concentrated using a rotary evaporator. Finally, the plant extract was dried in a vacuum oven at a temperature of forty degrees Celsius.

Animal Management

The mice were grouped and kept in polypropylene cages. They were provided with clean water and standard laboratory pellet meal. The temperature in the testing room was maintained at $22 \pm 3^{\circ}\text{C}$, with a relative humidity of 70%. A 12-hour dark / 12-

hour light cycle was followed. To administer the medication, an 18-gauge oral feeding needle was used.

Ethics Approval

The trials were run in accordance with national and international law, as mentioned in studies (Mughal et al. 2019; Dar et al. 2019; Ali et al. 2020; Mughal et al. 2020a; Mughal et al. 2020b; Mughal et al. 2022; Nauroze et al. 2023a; Nauroze et al. 2023b). This page contains no research involving human subjects conducted by any of the authors. All subjects in the study provided informed consent.

Animal Selection and Grouping

Twenty male Swiss albino mice, aged 8 weeks on average and weighing between 35 and 40 grams, were procured from GC University Lahore. Initially, all the animals were housed together for a period of seven days. Four groups were made by the randomly selection of animals;

Group I: Olive oil was provided to the control group.

Group II: BLR extract group, received oral daily dose of BLR extract of 200 mg/kg for 28 consecutive days

Group III: Diabetic group; a single dose of alloxan equals 150 mg/kg in 0.9% saline was injected intraperitoneal.

Group IV: Diabetic + BLR extract group; after induction of diabetes received oral daily dose of BLR extract of 200 mg/kg for 28 consecutive days.

Induction of Experimental Diabetes

To induce diabetes mellitus in experimental mice, a single injection of alloxan (150 mg/kg in 0.9% saline) was administered. Ten mice received an intraperitoneal injection of alloxan. After that, the mice were fasted and their blood glucose levels were measured. Mice with blood glucose levels exceeding 200 mg/dL were diagnosed with diabetes.

Biochemical Determinations

After twenty-eight days of therapy, blood samples were directly drawn from the heart, and mice were euthanized for chemical and histological examinations.

Estimation of Blood Glucose

The blood sample was collected from a vein in the tail of the mouse. Blood glucose levels of fasting mice were measured using the One Touch Basic Glucometer after a 6-hour fast on days 7, 14, 21, and 28.

Estimation of liver markers

The Reitman and Frankel method was used to estimate ALAT and ASAT levels, the Bowers method was used to determine ALP levels, and the Hubscher method was utilized to assess LDH levels. Total bilirubin levels were determined using modified dimethyl sulfoxide (DMSO), and total protein was measured using the Lowry et al. method. Catalase activity was assessed using the Luck protocol with some modifications, reduced glutathione (GSH) activity was measured using the Ellman

standard protocol, and Malondialdehyde bis-(dimethyl acetal) tetra ammonium (MDA) activity was investigated using the Okkava et al. method.

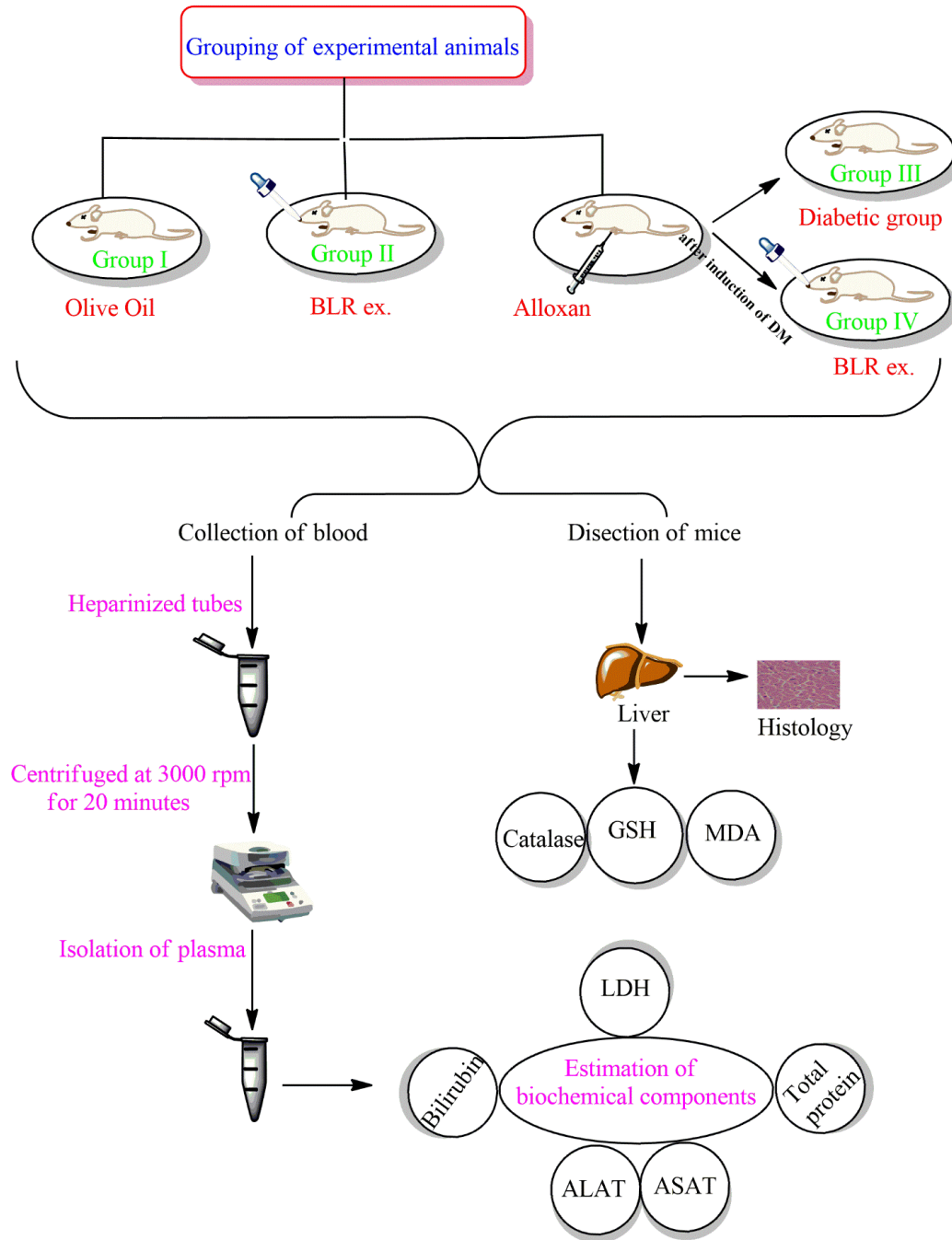


Figure 1: Diagrammatic representation of research plan.

Histopathological studies

For histopathological studies, buffered neutral formalin solution (10%) was used to fix the collected liver specimen. This liver specimen was dehydrated with the help of 70%–100% ethanol and was cleared with xylene. Then this sample was fixed in paraffin and paraffin sections of five-micron thickness were prepared. Microscopic

examination was done after staining the sample with dyes i.e. hematoxylin and eosin (HE).

Statistical analysis

We used GraphPad Prism to conduct all statistical analyses. The reported values were presented as mean $\pm$ SEM. To determine any statistical differences between groups, we performed the Bonferroni test of one-way ANOVA. Statistical significance was measured when $p < 0.05$.

RESULTS

Blood glucose level

The induction of diabetes using alloxan increased blood glucose levels. Aforementioned blood glucose changes were overturned after the curing diabetic mice for 28 days with 200 mg/kg root extract. The significant decrease in blood glucose level was recorded (Figure 2).

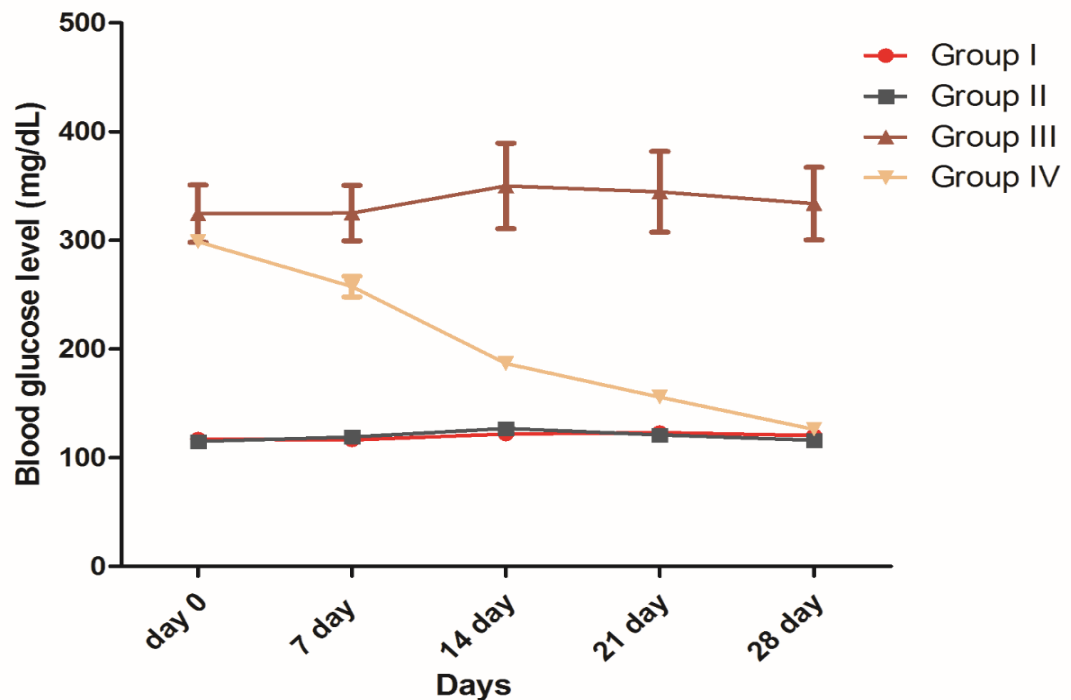


Figure 2: Graphical representation of changes in blood glucose level from day 0 to 28 of treatment.

Effect on ALAT

In the present study, we found that intraperitoneal administration of alloxan (150 mg/kg body weight) significantly increased the levels of ALAT. The control group had ALAT levels of 45.6 ± 3.7 IU/L, while the diabetic group had significantly higher levels of 162.4 ± 5.58 IU/L. However, when BLR extract was administered alone, it did not cause any significant change in ALAT levels (44.4 ± 1.27 IU/L) compared to

the control group (45.6 ± 3.7 IU/L). On the other hand, when mice with diabetes were orally administered with BLR extract at a dosage of two hundred mg/kilogram body weight, and has imperative reduction in ALAT levels (85.2 ± 3.41 IU/L) (Figure 3).

Effect on ASAT

In this investigation, we discovered that administering alloxan intraperitoneally at a quantity of 150 mg/kilogram body weight significantly increased ASAT levels. The ASAT levels in the control group were 80.2 ± 3.1 IU/L, whereas in the diabetic group, they reached 482.2 ± 15.4 IU/L. When the mice were given BLR extract alone, there was no notable difference in ASAT levels compared to the control group, which averaged at 85.8 ± 2.61 IU/L. However, when we administered BLR extract at a dosage of two hundred mg/kilogram body weight to diabetic mice, we observed a significant reduction in ASAT levels. In the diabetes + BLR extract group, the ASAT levels were measured at 164.4 ± 9.07 IU/L (see Figure 3).

Effect on ALP

The study discovered that injecting alloxan intraperitoneally at a dose of 150 mg/kg body weight led to a significant increase in ALP levels when compared to the control group (control: 113.4 ± 3.86 IU/L; diabetic: 312.2 ± 12.34 IU/L). However, when BLR extract was administered alone, there was no significant difference compared to the control group (113.6 ± 3.6 IU/L). Conversely, the oral administration of BLR extract at a dose of 200 mg/kg body weight to diabetic mice resulted in a significant reduction in ALP levels (diabetes + BLR extract: 231.2 ± 14.7 IU/L; Figure 3).

Effect on LDH

In this investigation, administering alloxan intraperitoneally (150 mg/kg body weight) significantly increased LDH levels (control: 349.3 ± 30.7 IU/L; diabetic: 955 ± 74.66 IU/L). When BLR extract was administered alone, there was no significant difference (339.4 ± 16.56 IU/L) compared to the control (349.3 ± 30.7 IU/L). When diabetic mice were given BLR extract orally (200 mg/kg body weight), their LDH levels decreased significantly (diabetes + BLR extract: 603.6 ± 28.38 IU/L) (Figure 3).

Effect on MDA

The study found that intraperitoneal administration of alloxan (150 mg/kg body weight) significantly increased MDA levels (control: 45 ± 4.02 mmol/g liver; diabetic: 672.2 ± 16.66 mmol/ gram liver). The extract of BLR was administered alone, it had no noteworthy effect (47.8 ± 5.03 mmol/gram liver) associated to the control (45 ± 4.02 mmol/ gram liver). However, giving diabetic mice 150 mg/kilogram body weight of BLR extract resulted in a considerable decrease in MDA levels (diabetes + BLR extract: 354.4 ± 29.68 mmol/ gram liver) (Figure 4).

Effect on GSH

The study found that intraperitoneal administration of alloxan (150 mg/kg body weight) significantly reduced GSH levels (control: 4.18 ± 0.12 μ mol/gram liver; diabetic: 2.24 ± 0.18 μ mol/gram liver). The extract of BLR was administered alone, it had no significant effect (4.16 ± 0.07 μ mol/gram liver) compared to the control

(4.18 ± 0.12 $\mu\text{mol/gram liver}$). However, giving diabetic mice the BLR extract orally (150 mg/kilogram body weight) caused in a considerable increase in GSH levels (3.68 ± 0.42 $\mu\text{mol/gram liver}$) (Figure 4).

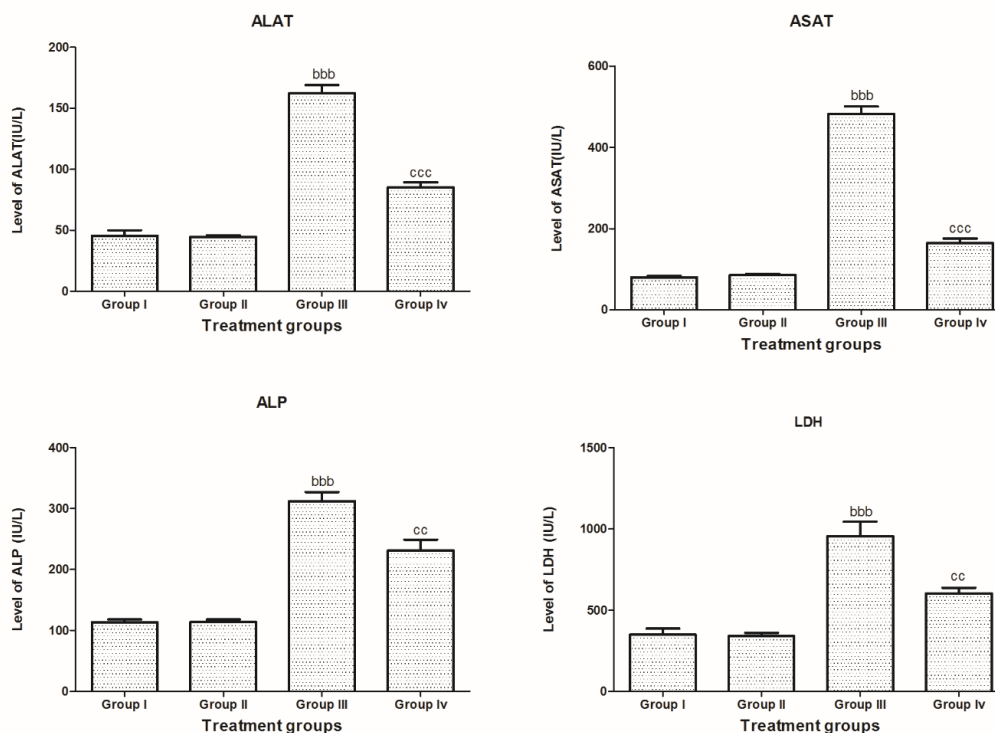


Figure 3. Analysis of enzymatic activity (ALAT, ASAT, ALP and LDH)
Significant difference in ALAT, ASAT, ALP and LDH was observed between group I (control) and group III (diabetic), group III (diabetic) and group IV (diabetic + BLR extract). ^adepicts difference between group I and group II. ^bdepicts difference between group I and group III treatment groups. ^cdepicts difference between group III and group IV. Each bar represents the mean value of five replicates and SEM. Statistical icons: cc= $p \leq 0.01$, bbb, ccc = $p \leq 0.001$

Effect on CATALASE

The study found that intraperitoneal administration of alloxan (150 mg/kg body weight) significantly reduced CATALASE levels (control: 186.4 ± 9.96 mmol/min/g liver; diabetic: 130.8 ± 6.62 mmol/min/g liver). When the extract of BLR was administered alone, it had no noteworthy effect (174.4 ± 9.71 mmol/min/g liver) compared to the control (186.4 ± 9.96 mmol/min/g liver). Giving diabetic mice 150 mg/kg body weight of BLR extract resulted in no significant change in CATALASE levels (161.4 ± 4.72 mmol/min/g liver) (Figure 4).

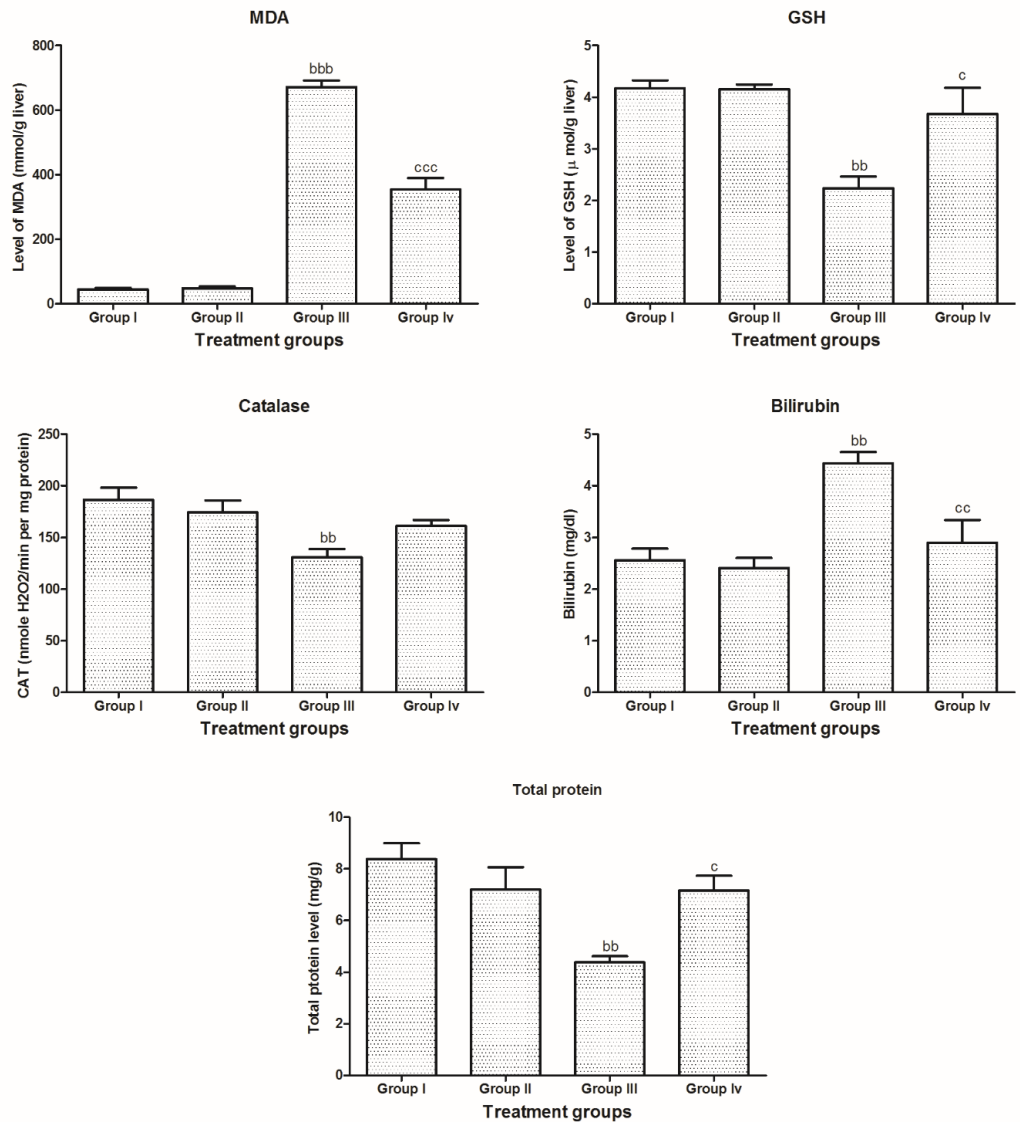


Figure 4: illustrates the analysis of MDA, GSH, CATALASE, total bilirubin, and total protein in Swiss albino mice.

Significant differences in levels of MDA, GSH, total bilirubin, and total protein were observed between groups I (control), III (diabetic), and IV (diabetic + BLR extract). There was also a significant variation in CATALASE between groups I as well as III. The figure clearly depicts the differences between groups I and II, as well as between treatment groups I and III, and between groups III as well as IV. Every bar represents five replicates the mean with SEM. The icons of statistics indicate significance levels: c indicates $p < 0.05$; bb, cc = $p \leq 0.01$, and bbb, ccc = $p \leq 0.001$.

Effect on total bilirubin

The study found that intraperitoneal administration of alloxan (150 mg/kg body weight) significantly increased bilirubin levels (control: 2.56 ± 0.18 mg/dl; diabetic: 4.44 ± 0.18 mg/dl). When the BLR extract was administered alone, it had no significant effect (2.41 ± 0.16 mg/dl) compared to the control (2.56 ± 0.18 mg/dl). However, giving diabetic mice 150 mg/kg body weight of BLR extract resulted in a considerable decrease in bilirubin levels (diabetes + BLR extract: 2.9 ± 0.37 mg/dl) (see Figure 4).

Effect on total protein

The study revealed that when alloxan was administered intraperitoneally at a dose of 150 mg/kg body weight, there was a significant reduction in total protein levels (control: 8.38 ± 0.5 g/dl; diabetic: 4.38 ± 0.19 g/dl). However, when the BLR extract was administered alone, there was no significant difference (7.2 ± 0.73 g/dl) compared to the control (8.38 ± 0.5 g/dl). Interestingly, when the BLR extract was orally administered at a dose of 150 mg/kilogram body weight to diabetic mice, there was a considerable increase in total protein levels (diabetes + BLR extract: 7.16 ± 0.48 g/dl; Figure 4).

Histology

After the completion of experimental trial, mice were dissected for liver sample. Sample were fixed and stained with H&E. Finally, observed to investigate the effect of alloxan and reversal of this condition by BLR-extract. Administration of alloxan caused damaged as seen in figure 5 (c) but BLR-extract prevented the cells from damage as visible in figure 5 (d).

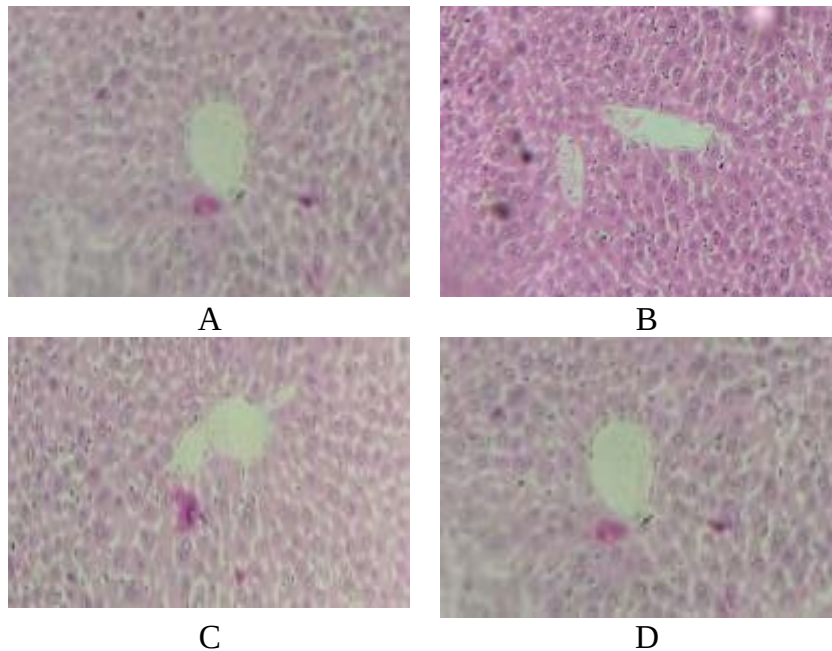


Figure 5: H&E stained liver cells. (A) Control (B) BLR extract (C) Diabetic (D) Diabetic+BLR-extract.

DISCUSSION

The administration of Alloxan to Swiss albino mice caused in a noteworthy growth in plasma levels of ALP, LDH, ALAT and ASAT compared to the control group. However, when diabetic mice were given *B. lycium* Royle extract, their plasma levels of ALP, LDH, ALAT and ASAT decreased significantly compared to the diabetic group in present study.

Studies have shown that injecting CCl₄ led to increased levels of LDH, ASAT, and ALAT in rats (Lin et al., 1993), Balb C mice (Mughal et al., 2019), and Swiss albino mice (Mughal et al., 2020a). Moreover, when rats were administered CCl₄ for 24 hours, there was an increase in plasma ALAT and ASAT activity, depletion of sulfhydryl, liver lipid peroxidation, genotoxicity, and a decrease in overall antioxidant capacities. CCl₄ also elevated levels of ASAT and ALAT in the liver, as well as lipid peroxidative enzymes such as catalase and superoxide dismutase (Bhattacharjee, 2006). These findings suggest that CCl₄ may cause liver tissue damage, leading to the release of these enzymes into the bloodstream. In a study where CCl₄ induced hepatotoxicity in mice, LDH levels and liver transaminases increased significantly compared to the control group. However, pretreatment with carrot extract resulted in a substantial reduction in LDH levels compared to the group treated with only CCl₄ (Jain et al., 2012).

According to Al-Snafi et al. (2019), mice with carbon tetrachloride-induced hepatotoxicity were treated with either 200 mg/kg b.w. of ethanolic or aqueous floral extracts of *Helianthus annuus*. The levels of ALP, ASAT, and ALAT were significantly reduced ($p < 0.001$) compared to the group treated with CCl₄, suggesting a decrease in oxidative stress caused by carbon tetrachloride. These findings support our own research. Similar results were observed in rabbits with carbon tetrachloride-induced hepatotoxicity when they were given 100 mg/kg b.w. of alcoholic root extracts of *Cynodon dactylon*.

Significant drops in levels of bilirubin and liver transaminase enzymes were observed, except in the case of CCl₄-treated rabbits where they increased. The current study discovered that in alloxan-intoxicated mice, levels of MDA and bilirubin increased while total protein, catalase, and GSH levels decreased. However, these symptoms were successfully reversed with the administration of the BLR-extract. These findings align with the findings of Al-Snafi (2017), who reported a notable reduction in SOD, GSH, and CAT levels in rats subjected to thioacetamide-induced oxidative stress.

Treatment of mice with *Dacus carota* seed extract led to a significant increase in levels of CAT, GSH, and SOD compared to the thioacetamide groups. In a study conducted by Rahate and Rajasekaran (2015), female rats treated with tamoxifen, which induce hepatotoxicity showed a noticeable rise in MDA levels and a decrease in total protein levels compared to the control group. However, the polyphenolic fraction of root of *Desmostachia bipinnata* reduced MDA levels while increasing GSH and total protein, and SOD, levels in the blood of female rats.

CONCLUSION

According to the results, the injection of alloxan into Swiss albino mice resulted in liver damage. Various biochemical parameters, including ALT, AST, ALP, LDH, GSH, MDA, catalase, bilirubin, and protein, were impacted. However, the extract from *B. lycium* Royle was able to restore these parameters to their normal levels. This suggests that *B. lycium* Royle extract has the impending to protect diabetic mice from alloxan-induced damage.

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CONFLICTS OF INTEREST

All authors declare there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Tafail Akbar Mughal and Shaukat Ali designed the study; Tafail Akbar Mughal conducted the experimentations; All authors analyzed the data and wrote and approved the manuscript.

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