ORIGINAL ARTICLE

Advances in Basic Medical Sciences

Hepatoprotective property of Berberis lycium in paracetamol induced liver injury; An Experimental study

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ABSTRACT

Objective: The aim of current study is to find out the Hepatoprotective effects of berberis lycium in paracetamol induced hepatic injury in mice.

Methodology: This research was carried out in Peshawar Medical College (PMC) Peshawar Pakistan and Kabir Medical College Peshawar. A sample of 30 mice was subdivided in to 6 groups of 5 mice each. Group I was Positive control group and given Silimyrin 50 mg/kg body weight followed by Paracetamol 250 mg/kg/body weight after three hours. Group II was Negative control group and Group III was Normal control group where Paracetamol 250 mg/kg body weight and Normal Saline was given respectively. Group IV, V, VI were given plant extract at respective doses of 100, 200, 400 mg/kg body weight, each followed by Paracetamol 250 mg/kg/body weight after 3 hours. Blood was extracted for determination of ALT, ALP, serum and total protein using standard protocols while tissue samples were isolated for histopathological studies.

Results: The test drug produced dose dependent significant decrease in levels of enzymes under study as compared to control while levels of T-protein were significantly increased as compared to control. Furthermore, histopathological studies reveals that the extract of Berberis lycium shows Hepatoprotective effects in dose dependent manners.

Conclusion: This study reveals that Berberis lycium shows Hepatoprotective properties.

Key words: Paracetamol, Acetaminophen, Hepatic injury, berberis lycium, Hepatoprotective properties

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INTRODUCTION

Acetaminophen (N-acetyl-para-aminophenol), also known as APAP or Paracetamol, is one of the most widely used agent for its anti-pyretic and analgesic properties since 1955 due to its easy availability as "over-the-counter medicine".[1] It is safe at a therapeutic dose of 4gm/day in adults. Paracetamol overdose either intentionally or unintentionally is the leading cause of acute fulminant hepatic failure all over the world.[2] In total poisoning cases registered in UK and USA, paracetamol poisoning contributes 50% and 10% cases respectively.[3] Another data reported 44% self-poisoning cases of paracetamol[4], whereas such data is lacking in Pakistan but seemingly the condition might be worse. Paracetamol, after oral administration is rapidly absorbed from the intestine and metabolized in liver. About 2% of the drug is eliminated unchanged in urine while 10% of the drug is pushed to phase I oxidation where a very lethal intermediate N-acetyl-para-benzo-quinone imine (NAPQI) is formed by several P450 cytochrome enzymes.[5] In human, rats and mice, NAPQI is converted to a less toxic compound principally by conjugating it with reduced glutathione to form 3-glutathione-S-yl-paracetamol conjugate, with the help of spontaneous or glutathione-S-transferase (GST) mediated conditions. The toxic effects of paracetamol on liver are mainly due to the production of NAPQI that develops as a consequence of oxidative capacities of this highly reactive and toxic metabolite and is related to oxidative stress. Furthermore, because of variable alterations in the glutathione disulfide (GSSG) and by the process of reduction in the activities of the antioxidant enzymes glutathione reductase, the paracetamol-associated

This article may be cited as: Shah FA, Parveen T, Shah HA, Naseem R, Iqbal W. Hepatoprotective property of Berberis lycium in paracetamol induced liver injury; An Experimental study. Adv Basic Med Sci. 2020;4(2): 69-75

depletion of the intrahepatic GSH occurs.[6,8] Other enzymes that also take part in depletion of intrahepatic depletion includes g-gluramyl cysteinyl synthetase, catalase, glutathione peroxidase and superoxide dismutase.[9,10] Once membrane of mitochondria is damaged, the mitochondria lacks the capacity to synthesize ATP, and ATP depletion causes cell death.[11-12] Massive necrosis of the liver cells is the most prominent feature of the acetaminophen toxicity.[13]

Currently, the only FDA approved anti-dote to counter paracetamol poisoning is N-Acetyl cysteine (NAC) that rapidly corrects the GSH deficiency and detoxify NAPQI but is only beneficial in early stages. Furthermore, adverse events like anaphylactic and gastrointestinal reactions limits its use with cautions.[14,15] Activated charcoal is also used to limit paracetamol absorption from the gut[16] but is ineffective in later stages. Therefore, much research is needed to develop safe and effective agents to combat paracetamol poisoning and hepatotoxicity.

Berberis lycium belongs to family Berberidaceae, possess different pharmacological activities including anti-diabetic, anti-hyperlipidemic, anti-diarrheal, anti-spasmodic, anti-protozoal, anti-bacterial and hepato-protective effects. This study focuses on the hepato-protective effect.[17,18] of Berberis lycium on paracetamol induced hepatic injury in mice.

MATERIALS AND METHODS

STUDY DESIGN AND SETTINGS

It was quasi-experimental study conducted of healthy and adult swiss albino mice of either gender weighing 25-35gm. The animals were maintained at standard laboratory conditions (25 ± 2 °C and 12 hours light - 12 hours dark cycles) and were fed with standard food and water. The experimental protocols for the animal studies were approved by the ethical committee of the Riphah International university and Peshawar Medical college, Pakistan. All the experimentation was performed in pharmacology laboratory PMC and Kabir Medical College Peshawar.

CHEMICALS

The required chemicals include Paracetamol and diagnostic kits for the determination of alkaline phosphatase (ALP), alanine aminotransferase (ALT), serum bilirubin and total protein were obtained from Sigma Aldrich Germany.

Plant collection and extract preparation

Plant was collected from various parts of Lower Dir and Swat, Khyber Pakhtunkhwa Pakistan and identified by qualified botanist in the department of botany, Islamia University Peshawar. The leaves were shade dried and grinded into fine powder. 1kg of plant material was soaked in 8-liter methanol for 72 hours with occasional stirring which was then passed through filter paper. The extract was prepared with rotary evaporator at 40oC to obtain dark brown paste.

EXPERIMENTAL WORK

Swiss albino adult mice (25-35gms) of either sex were part of this study. The mice were obtained from Veterinary Research Institute Peshawar and were placed in the animal house of PMC. Standard environmental conditions were maintained including maintenance of temperature at 25±2°C with dark and light cycle (12/12 hours). The period of acclimatization lasted for about 10 days with standard diet.

ACUTE TOXICITY TEST

To determine the LD50 of the crude extract, the adult healthy mice were randomly divided into 7 groups with 6 animals in each group. Group 1 serves as control which were given normal saline while different doses of Berberis lycium extract were given in ascending order to group 2, 3, 4, 5, 6 and 7 at dose of 200, 400, 600, 800, 1000 and 1200mg/kg respectively. The mortality rate was observed for 24 hours. The LD50 was calculated according to previous work published.

EXPERIMENTATION

For experimentation, the 30 animals were subdivided into six different groups, each having 5 mice. Group I was Positive control group and given Silymarin 50 mg/kg body weight followed by Paracetamol 250 mg/kg/body weight after three hours. Group II was Negative control group and Group III was Normal control group where Paracetamol 250 mg/kg body weight and Normal Saline was administered respectively. Group IV, V, VI were given plant extract at dose of 100, 200, 400 mg/kg body weight, each followed by Paracetamol 250 mg/kg/body weight after 3 hours. Animals were slaughtered by cervical decapitation on day ten and blood was collected for biochemical investigations.

BIOCHEMICAL INVESTIGATIONS

The blood were subjected to centrifuge for serum isolation at 4000 cycles/min for about 20 minutes and Liver Function tests (LFTs) i.e. ALT, ALP, Serum bilirubin and total protein were estimated as per standard protocol.

Table 1: Effect of Berberis lycium as compared to control

S. No.	Treatment	ALT ± SD	ALP ± SD	Serum bilirubin± SD	Total protein ± SD
1	Saline	39.40 ± 5.06	247.60 ± 14.55	1.00 ± 0.03	3.88 ± 0.23
2	Silymarin + paracetamol	224.00 ± 8.12 ***	146.80 ± 37.95 ***	0.58 ± 0.03 ***	5.42 ±0.18 **
3	Paracetamol –ive	571.40 ± 61.63 ***	518.00 ± 20.59 ***	1.92 ± 0.13 ***	4.34 ± 0.18 **
4	Experimental Group (100mg/kg)	232.00 ± 21.13 ***	262.00 ± 38.91 ***	0.80 ± 0.08 ***	5.66 ± 0.24 **
5	Experimental Group (200mg/kg)	143.00 ± 16.25 ***	184.60 ± 23.42 ***	0.82 ± 0.05 ***	5.52 ± 0.11 **
6	Experimental Group (400mg/kg)	62.20 ± 9.08 ***	155.00 ± 17.18 ***	0.84 ± 0.06 ***	5.72 ± 0.28 ***

* P <0.05, ** P < 0.003 and *** P <0.001, ALT: alanine aminotransferase, ALP: alkaline phosphatase

HISTOPATHOLOGICAL STUDIES

Liver from the animals were isolated and subsequently stored in buffered formalin solution (10%). The samples were embedded in paraffin wax after dehydration with graded alcohol dilutions followed by xylene treatment. The microtome were used to cut 5-6µm sections and subsequently stained with hematoxylin and Eosin (H&E) staining was done using the protocols previously mentioned. The slides were examined under microscope with 10x magnification for histopathological changes.

STATISTICAL ANALYSIS

All the values are expressed in mean ± standard deviation (SD). One way analysis of variance (ANOVA) was utilized as judgment test of significant difference among groups followed by Dunnet's multiple comparison Post-test using statistical package for social sciences (SPSS) version 22.0. P-values less than 0.05 were considered significant.

RESULTS

HEPATOPROTECTIVE ACTIVITY

The test drug produced a dose-dependent decrease in the levels of enzymes under study. This was signified by a progressive decrease in SGPT of the test drug at the dose of 100 mg/kg (P < 0.001), 200 mg/kg (P < 0.001) and 400 mg/kg (P < 0.001) as compared to control. Similarly, the levels of ALP and SBR significantly decreased at the tested doses of 100 mg/kg (p < 0.001), 200 mg/kg (P < 0.001) and 400 mg/kg (P < 0.001) as compared to control. Whereas, the T-protein levels were increased at the tested doses 100 mg/kg (P < 0.01), 200 mg/kg (P < 0.01) and 400 mg/kg (P < 0.001) as compared to control. Table 1 below summarizes the results.

We also studied the histopathological changes that occurred in different groups, i.e., experimental and control groups. In figure 1 below legend 1a shows normal histology of liver. This was normal control group in which only normal saline was given for about 9 days. No histopathological changes were observed in this group. Portal triads are visible and there is no evidence of hemorrhages or necrosis.

In the negative control group (legend 1b), which was given only acetaminophen (250 mg/kg body weight) for a period of 9 days. There are obvious signs of damage to the liver cells. There are microcellular changes in the cells. Nuclei of the cells have been washed out (black arrow) and fatty changes (white arrow) are clearly visible. In portal triad, there is infiltration of inflammatory cells (blue arrow).

Furthermore, in positive control group in which silymarin (50 mg/kg body weight) along with acetaminophen (250 mg/kg body weight) after 3 hours gap was given. There was less damage to the cells. There was zone wise damage, minute amount of hemorrhages (arrow) and portal triad was visible with no fatty changes (small arrow: legend 1c).

The experimental group E1 in which acetaminophen (250 mg/kg body weight) along with extract of plant (Berberis lycium) in dose of 100 mg/kg body weight was given, showed focal hemorrhages (**encircled**) with no microcellular damage as was present in negative control (legend 1d). This showed that plant Berberis lycium has Hepatoprotective property.

In extract group E2 we have only increased the dose of plant extract to 200 mg/kg body weight. There were minute focal hemorrhages (long arrow) as compared to the previous group E1. Portal triad (short arrow) was visible with no **microcellular changes. This again shows the** hepatoprotective effects of Berberis lycium (legend1e). In extract group E3, the dose of plant Berberis lycium has been

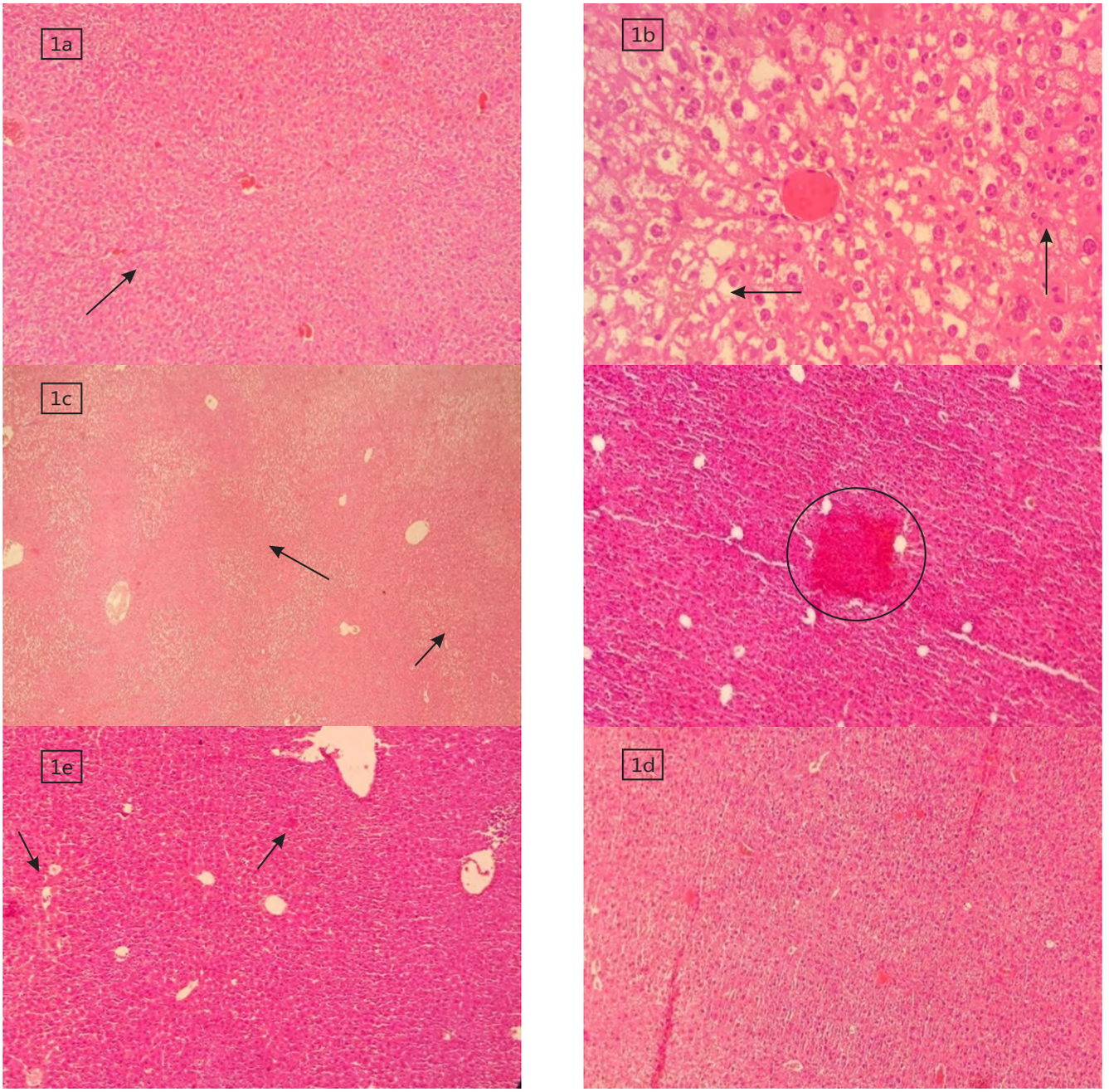

Figure 1: Histology of mice liver

1a: Histology of mice liver in normal control group
1b: Histology of mice liver in negative control group
1c: Histology of mice liver in positive control group
1d: Histology of mice liver in plant extract group E1 (100 mg/kg body weight)
1e: Histology of mice liver in plant extract group E2 (200 mg/kg body weight)
1f: Histology of mice liver in plant extract group E3 (400 mg/kg body weight)

increased to 400 mg/kg while dose of paracetamol was same (250mg/kg). In this group no damage to the liver was observed in the slide. This confirms the Hepatoprotective properties of Berberis lycium (legend1f).

DISCUSSION

The adverse effects of drugs have been a matter of serious concern to a prescriber. Even the drugs that were tested and proved to be less toxic were found to be responsible for many adverse effects in the post marketing surveillance phase and for the same reason have been withdrawn from the market. The fact that liver plays important role in metabolism of many drugs and chemicals predisposes the liver directly to face a major share of the overall damage caused by these drugs. To safeguard the liver from these damages many drugs have been developed and there is always a need for a better drug in this regard. The unique anatomical position of liver between the portal and hepatic circulation causes it to face a wide variety of different substances which are metabolized and eliminated by the liver with the help of wide array of different enzyme systems. But concentration of these chemical substances matters which might damages the liver because in normal situation the liver has the capability to cope-up these situations. Among these chemical reagents/drugs, paracetamol also damages the liver in high concentration and this is the fact that for evaluation of the Hepatoprotective properties of new agents, paracetamol induced liver toxicity is frequently used in animal model.

Berberis lycium is a plant which has been mentioned in folk medicine for its healing properties including beneficial effects on liver. This became the drive for the present study because no such scientific study has been previously performed to prove the claims about the usefulness of Berberis lycium in liver aliments. Berberis lycium extract was screened for its Hepatoprotective effects by the available in vivo test model system which has its own limitations and is feasible only with hepato-toxins, producing reproducible type of hepatic injury, capable of conveniently quantified.

Various research scholars have evaluated herbal plants for their antioxidant properties which help in protecting various organs from oxidative damage. Paracetamol induced hepatotoxicity was proved by means of biochemical and histopathological evidence in this study. Berberis lyceum decreases the increased levels of the ALT, ALP, and serum bilirubin most significantly and increases the total protein insignificantly. The effect on serum ALT levels is the most obvious finding; it is higher than that produced by the standard drug silymarin. This shows that the hepatocytes are better protected by this drug (as shown). On the basis of histological and pathological studies, severe wear and tear in hepatocytes was documented in paracetamol treated mice. Rafiq et al, used bark of Berberis lycium against isoniazid-induced liver toxicity and found the hepatoprotective activity of the plant. A similar study was conducted by Khan and his colleagues where hepatoxicity in rats were induced with carbon tetrachloride (CCL), reported significant difference in ALT, AST and ALP levels between CCL4 treated and Berberis lycium treated group ($p<0.001$). Studies of antioxidant properties of Berberis lycium have been conducted where researcher observed that the plant extract inhibits the formation of free radicals which damage the liver and cause fibrosis. So, this plant can be used in jaundice being possessing anti-oxidant activity as well.

This study shows that the methanolic extract of leaves of Berberis lycium in higher doses most significantly ($p<0.001$) reversed the inflammatory changes in mice liver. The histopathological changes induced by acetaminophen got improved by the extract which may be attributed to the presence of substances such as flavonoids and berberine in leaves of berberis lycium. Because of its several beneficial properties such as edible nature, easy accessibility and cost effectiveness, berberis lycium can be regarded as a beneficial source of active contents capable of healing hepatic ailments.

Study limitations

Different model of hepatotoxicity might be used to authenticate the efficacy of berberis lycium further. Furthermore, compound isolation needs to carried out to determine the actual compound that shows hepatoprotective activity.

CONCLUSION

Berberis lycium shows Hepatoprotective effects on paracetamol induced hepatotoxicity in dose dependent manner. The maximum effect is achieved at 400mg/kg.

Conflict of interest

The authors declare no conflict of interest.

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