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Reduction of liver enzyme ALT by berberis lycium in acetaminophen induced hepatic damage in mice liver.

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ABSTRACT… Objective: The objective of this research was to study the effect of Berberis lyceum (herbal plant) in reduction of liver enzyme ALT in mice. **Study Design:** Quasi-experimental Study. **Setting:** Peshawar Medical College, Peshawar. **Period:** December, 2016 to May, 2017. **Materials & Methods:** It was an experimental study which was carried out at Peshawar Medical College Warsak Road Peshawar. Total 30 (thirty) mice were used divided in six different groups with five mice in each group. After inducing hepatotoxicity with acetaminophen in the mice, blood samples were taken and LFTs were performed to observe serum ALT values and the effects of different doses of the plant extract were evaluated. **Results:** The ALT levels of negative control and experimental group were compared and it was shown that ALT levels in experimental groups were 232±42.3, 143±32.5 and 62.2±18.2 whereas serum ALT value of negative control group was 571.4±123.3 which showed that ALT had been decreased by increasing the dose of plant extract. **Conclusion:** It was concluded that Berberis Lycium, a herbal plant has a potentially active hepato protective role in bringing serum ALT to normal. This plant can be useful in reversing the hepatotoxicity in drug induced elevation of liver enzyme ALT.

Key words: ARD- Adverse Drug Reactopn, CCL4- Carbon Tetrachloride, SD- Standard Deviation.

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INTRODUCTION

Health and disease have been a subject of man's primary concern since ancient times. From his early experiments with herbs and plants growing around his environment and using them in various diseases, man was eventually able to establish empirical system of medicine. A large proportion of human population is on herbal remedies. It has been established by WHO that about 80% of the world population rely mostly on traditional medicines.[1]

Phytochemical examinations of plants and animals cure available have often shown that these contain active principles, which produce responses for their therapeutic success. Moreover, empirical studies on medicinal plants revealed the fact that for a plant extract to be active clinically, it is not necessary for the active components to be isolated and structure established. A large number of crude plant extracts are now being utilized in naturopathic remedies in additional to the purified natural substances.[2]

Liver is the main organ related to the metabolism of a large number of drugs and pollutants. Morphologically mouse liver, weighs approximately 1.3 to 1.5 grams.[3]

Liver is responsible for the detoxification of and metabolism of various drugs, chemicals, metals, bilirubin, steroid hormones and amino acid.[4,5]

Liver is involved in about 80% of reports of ADRs. It is because of the major role in the biotransformation and elimination of many drugs. The list of drugs implicated in drug-induced liver disease is extensive. Acetaminophen is most commonly used as an analgesic and it's over dosage either intentionally or unintentionally

remains the most common cause of fulminant hepatic failure with mortality rate of 90%.[4] This is because of higher dose acetaminophen's conversion to extremely toxic intermediate N-acetyl-p-benzoquinone imine (NAPQI) by several P450 cytochrome enzymes.[6]

Berberis lycium (family: Berberidaceae) is a plant whose leaves are used as hepatoprotective remedy in folk medicine since time immemorial.[7] It is mostly found in the Himalayan region. In Pakistan, it grows in Baluchistan, Khyber-Pakhtunkhwa, Punjab and Azad Kashmir at a height of 900 to 2900 meters.

Leaves of Berberis lycium are bright in color which makes it attractive in appearance. The Leaves are oblanceolate to oblong-ovate shape, thick and entire toothed. Berberis lycium leaves .are commonly used for the treatment of various disorders in the liver due to its antioxidant properties. They normalize the deranged liver function, for jaundice as a tea substitute.[8]

There are various pharmacological effects of plant Berberis lycium which make it clinically important. Berberis lycium is reported to have many unique properties and most important of them are antibacterial, aperients, anti-carcinogenic, carminative, and febrifuge.[9]

Berberis lycium is reported to have certain pharmacological effects also, such as anti-diabetic and anti hyperlipidemic.[10] It has also been reported that Berberis lycium is extensively used for medicinal purposes as for example folk remedy for various endocrine abnormalities.[11] The rationale of the study is to observe the hepatoprotective role of Berberis lycium as it needs to be scientifically proved so that some commercial drugs may be developed from this plant to benefit the mankind based on the fact that Berberis lycium contains in it curative effects more than preventive.[9,10]

AIMS AND OBJECTIVES

To determine hepatoprotective activity of Berberis lycium on acetaminophen-induced mice liver damage by observing serum ALT levels.

METHODS & MATERIALS

It was a Quasi- experimental Study conducted at Peshawar Medical College, Peshawar for Six Months from December, 2016 to May, 2017.

Thirty (30) Swiss albino mice were selected Randomly.

Inclusion Criteria

Healthy young adult mice of either sex.
Age of mice 8-12 weeks.
Weight of each mouse 22-35 gm.

Exclusion Criteria

Pregnant female mice
Diseased mice

The plant Berberis lycium was selected on the basis of its traditional use and its phytochemical profile. Leaves were picked out from the stems, washed and then were placed in shade to dry. It took about 15 to 20 days to get them dried. After that, the leaves were grounded in to powder with the help of a grinder. Powdered leaves were then soaked in 8 liter of 99.9% methanol for about 7 – 10 days. This paste was shaked regularly on alternate days. On the day 10, the solution was filtered with the help of muslin cloth. Filtrate was collected in to conical flasks and was allowed to evaporate with the help of the rotary evaporator. It took about 3 days and at last a brownish green paste was obtained which was placed in a dish. The dish was kept in water bath to let it dry like a thick jell for 4 days. The jell paste was stored in amber colored bottle for further proceedings.

Mice were placed in animal house of Peshawar Medical College. These mice were housed in cages under standard environmental conditions (temperature 25±2°C) with dark and light cycle (12/12 hours). They were acclimatized for a period of about 10 days.[11] They were fed on standardized pellet diet with water ad libitum.

All the thirty animals were divided into six different groups with five animals each.
Group I. Positive control group. (Silimyrin 50 mg/kg body weight was given and after 3 hour

Acetaminophen 250 mg/kg/body weight was given)
Group II. Negative control group. (Only Acetaminophen 250 mg/kg body weight was given)
Group III. Normal Control group. (Normal Saline was given)
Group IV. Plant extract Group E1. (Plant Extract was given 100 mg/kg body weight and then after 3 hours Acetaminophen 250 mg/kg/body weight was given)
Group V. Plant Extract Group. E2 (Plant Extract was given 200 mg/kg body weight and then after 3 hours Acetaminophen 250 mg/kg body weight was given)
Group VI. Plant Extract Group. E3 (Plant Extract was given 400 mg/kg body weight and then after 3 hours Acetaminophen 250 mg/kg body weight was given).[12,13]

The duration of the study was ten (10) days after which the mice having remained fasted for 12 hours, anesthetized with light chloroform and by cervical decapitation.[14] Blood was collected by direct puncture in to aorta and samples were preserved in tubes for serum preparation. Hepatotoxicity was indicated by a significant elevation in the activity of serum ALT in acetaminophen- challenged mice compared with the normal control group though out the experimental analysis.[15]

Blood after collection from the mice, was centrifuge for the separation of serum at the rate of 4000 cycles/min for about 20 minutes. Liver Function tests i.e., ALT was evaluated through standard operating procedures.[16]

RESULTS

The data of the variable ALT values in diverse study groups was collected and a series of statistical tests were performed on it. In the test of normality the whole data was found normally distributed. For significant results among different variables student t test was applied through SPSS version 20. For detailed analysis of variables, data was divided into two broad categories and compared.

- Control group
- Experimental group

Control group was further divided into normal control, positive control and negative control while experimental group into Plant Extract 1 (100 mg/kg), Extract 2 (200 mg/kg) and Extract 3 (400 mg/kg) groups.

In normal control group we gave only normal saline for the 9 days and after that slaughtered the mice on 10th day. Blood samples analysis showed the mean and SD of ALT (Alanine Aminotransferase) levels as 39.4±10.1 U/L.

In positive control group silimyrin (50 mg/kg body weight) was given and after 3 hours of silimyrin, acetaminophen (250 mg/kg body weight) was given, blood samples on day tenth showed ALT values to be 224±16.2 U/L.

In negative control group only Acetaminophen (250 mg/kg body weight) was given for 9 days and after that same procedure was applied , blood samples were collected and the mean and SD of ALT was 571.4±123.3 U/L as depicted in Figure-1.

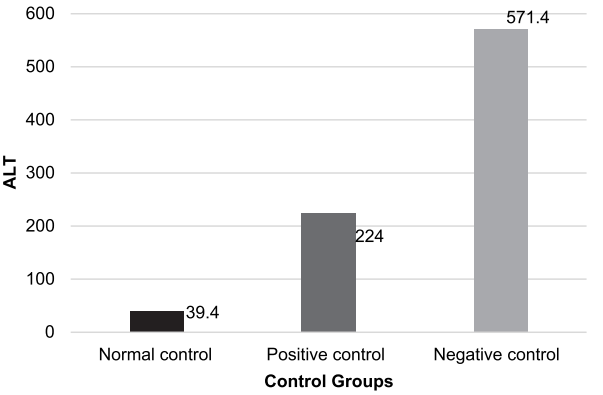

Figure-1. Control groups for ALT

Different doses of plant extract along with acetaminophen 250 mg/kg body weight (with 3 hours gap), were given and the values of ALT were noted. It was seen that with increasing the doses of Berberis lycium extract the serum ALT levels dropped as shown in the Figure-2. In E1. 100 mg/kg plant extract was given and serum ALT was 232±42.3 U/L, in E2. 200 mg/kg plant extract was given and serum ALT was 143±32.5 U/L and in E3. 400 mg/kg plant extract was administered

and serum ALT was 62.2±18.2 U/L. Figure-2.

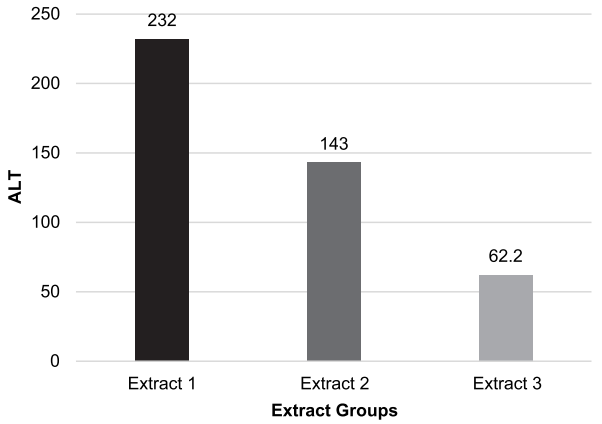

Figure-1. Control groups for ALT

DISCUSSION

Drug toxicity has been a matter of serious concern to medical practitioner. Many drugs that are reported to be toxic even in post-marketing surveillance, have been de-registered and as a consequence are now removed from the market. Liver being a major site of metabolism for many xenobiotics experiences the effect of injury the most. Although several drugs have been developed which protect the liver from such toxicities. However, there is always a need for a better drug in this respect.

In our study we used mice as animal model and induced hepatotoxicity in them with acetaminophen. Omidi and his coworkers used acetaminophen to induce liver toxicity in rats and then gave extract of Crocus sativa (saffron) to counter the toxicity.[17] Mallhi also studied the hepatoprotective effect of Morus nigra in acetaminophen –induced liver toxicity.[14] Both these research studies favor our study for the use of acetaminophen as hepatotoxic agent in high doses.

Literature review shows that many plant extracts have been studied to know about their hepatoprotective potential. Berberis lycium is one such plant which has been revealed in folk medicine for its healing properties including beneficial effects on liver. This became the motivation for the present study because no such scientific study has been previously done to prove the claims about the usefulness of Berberis lycium in liver ailments.

Berberis lycium extract was also screened for its hepatoprotective properties by the available in-vivo test model system, however this in-vivo study has its own limitations. In contrast, our research was quasi-experimental study of six months duration which included thirty healthy adult mice of either sex, aged 8-12 weeks weighing between 22-35.

In present experimental study, acetaminophen-induced hepatotoxicity was obvious through biochemical test findings. Berberis lycium decreases the elevated levels of serum ALT levels most significantly ($p<0.000$).

Many research scholars have studied herbal plants for their antioxidant properties important for protecting various organs from oxidative damage.

Rafiq with his colleagues conducted a research study using bark of Berberis lycium against isoniazid-induced liver toxicity and found the hepatoprotective activity of the plant. This study is in consistance with our study though we used leaves of the plant instead of bark.[15]

Girish in 2009 used extracts of Berberis lycium, Pistacia integerrima and Gallium aparine and found them beneficial in mice CCl_4-induced liver toxicity. The result of this study indicated that a mixture of these plants decreased the raised ALT and also reduced the process of necrosis in liver cells.[13] Their work supports our study for contributive hepatoprotective role of Berberis lyceum in combination of three herbs.

Berberis lycium has shown hepatoprotective effect in another experimental study conducted by Purvika and his coworkers in rats which were given carbon tetra chloride. The ALT was reduced to normal after treatment with Berberis lycium.[8] The contradiction to our study is that in their study Purvika used rats rather than mice as their experimental model. Furthermore he induced hepatotoxicity in rats by giving carbon

tetra chloride solution and then Berberis lycium was given.
A similar study was conducted by Khan and his colleagues (2011) but again they used rats as their experimental model and observed beneficial results.[9] Another study supportive to ours was conducted by Abbasi and his research fellows, where they found that this plant can be used in hepatitis due to its antioxidant properties. [18]

Antioxidant effect of Berberis lycium were also proved by Ahmed and Shakil in 2012 and UrRehman in 2013 also observed the same.[19,20] All of them showed that the plant significantly decreased the raised ALT levels near to normal; their observation is also in relevance to our study.

In light of above findings of our study and its concordance with the previous studies, it is indicated that the methanolic extract of leaves of Berberis lycium in higher doses significantly ($p<0.000$) reversed the inflammatory changes in mice liver. Due to its edible nature, easy accessibility and economical factors Berberis lycium can be a good source of active contents having healing potential in liver aliments. However, to add to the list of hepato protective drugs, further studies are suggested to know the active ingredients, their pharmacodynamics, pharmacokinetics and toxicity.

CONCLUSION

From the data analysis it is concluded that Berberis lycium has hepatoprotective properties. The herbal plant has the ability to decrease the increased levels of serum ALT most significantly. With increasing doses of the plant extract the liver functions nearly came to normal. The study results support the traditional use of this herbal plant.

CONFLICTS OF INTEREST

There is no conflict of interest in this research.

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