ORIGINAL ARTICLE

Effect of Berberis Lycium on Liver Enzymes ALT and ALP in Acetaminophen induced Hepatic Damage in Mice S

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ABSTRACT

Background: The use of medicinal plants in dealing diverse ailments is of remote antiquity. A large number of herbal medications are used as hepatoprotective agents. Berberislycium, a herbal plant has potential role in protection against hepatic damage.

Objectives: To study the hepatoprotective effects of Berberislycium in mice.

Methods: It was an experimental study, 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 for LFTs, serum Alanine transferase (ALT) and Alkaline phosphatase (ALP) values were recorded and the effects of different doses of the plant extract were evaluated.

Results: The Alanine Transferase ALT levels of negative control and experimental group were compared and it was seen that ALT levels in experimental groups were 232±42.3, 143±32.5 and 62.2±18.2whereasserum 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. Alkaline Phosphate ALP levels of negative control and experimental groups were compared and it was seen that ALP levels of experimental groups were 262±77.8, 184.6±46.8 and 155±34.4. On the other hand ALP of negative control group was 518±41.2, which showed that by increasing the dose of Berberislycium ALP had been decreased.

Conclusion: It was concluded that Berberis Lycium, a herbal plant has a potentially active hepatoprotective role in bringing liver enzymes serum ALT and ALP to normal. This plant can be usefulin reversing the hepatotoxicity in drug induced elevation of both ALT and ALP.

Keywords: BerberisLycium, ALT, ALP

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]

Berberislycium (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 Berberislycium are bright in color which makes it attractive in appearance. The Leavesareoblanceolate to oblong-ovate shape, thick and entire toothed. Berberislycium 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 asa tea substitute.There are various pharmacological effects of plant Berberislycium which make it clinically important. Berberislycium is reported to have many unique properties and most important of them are antibacterial, aperients, anti-carcinogenic, carminative, and febrifuge.7. Berberis Lyceium has a potential role in

treating many neurological disorders. 8 certain pharmacological effects also seen such as anti-diabetic and anti hyperlipidemic.[9] The hepatoprotective properties of *Berberislycium* needs to be scientifically proved so that some commercial drugs may be prepared from this plant to benefit the mankind.

Aims and Objectives: To determine hepatoprotective activity of Berberislycium on acetaminophen-induced mice liver damage.
Study Design: Quasi- experimental Study
Setting: Peshawar Medical College, Peshawar
Duration of Study: Six Months
Sample Size: Thirty (30) Swiss albino mice
Sample Technique: Random
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

METHODS AND MATERIALS

The plant Berberislycium was selected, Leaves were picked out from the stems, washed and placed in shade to dry. After 15 to 20 days the dried leaves were grounded to powder form . Powdered leaves were soaked in 8 liter of99.9% methanol for about 7 – 10 days. This paste was shaked regularly on alternate days. On the day 10,the solution was filtered with muslin cloth. Filtrates were collected in the conical flasks and were allowed to evaporate with the help of the rotary evaporator. After 3 days a brownish green paste was obtained , placed in a dish which was kept in water bath for 4 days to let it dry like a thick jell. That jellpaste was stored in amber colored bottle for further proceedings.

Thirty Mice were kept in cage in animal house of Peshawar Medical College. Standard environmental conditions (temperature 25±2°C) with dark and light cycle (12/12 hours) were maintained They were acclimatized for a period of about 10 days.[10]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: Normal Control group. (Normal Saline was given)
Group II: Positive control group. (Silimyrin 50 mg/kg body weight was given and after 3 hour Acetaminophen 250 mg/kg/body weight was given)
Group III: Negative control group. (Only Acetaminophen 250 mg/kg body weight was given)
Group IV: Plant extract Group E1. (Plant Extract was given 100mg/kg body weight and then after 3 hours Acetaminophen 250mg/kg/body weight was given)
Group V: Plant Extract Group. E2 (Plant Extract was given 200mg/kg body weight and then after 3 hours Acetaminophen 250mg/kg body weight was given)
Group VI: Plant Extract Group. E3 (Plant Extract was given 400mg/kg body weight and then after 3 hours Acetaminophen 250 mg/kg body weight was given).

The duration of the study was ten(10) days after which the mice were fasted for 12 hours, anesthetized with light chloroform and sacrificed by cervical decapitation. Blood was collected by direct puncture in to aorta and samples were preserved in tubes for serum preparation. 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 and ALP was evaluated through standard operating procedures.

RESULTS

The data of the variable ALT and ALP 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

Mean and SD in Group I serum ALT level was (Alanine Aminotransferase) levels was 39.4±10.1 U/L. Group II it was 224±16.2 U/L. and in group III it was 571.4±123.3 U/L while experimental group into Plant Extract 1 (100mg/kg), Extract 2 (200mg/kg) and Extract 3 (400mg/kg) groups. figure (1).

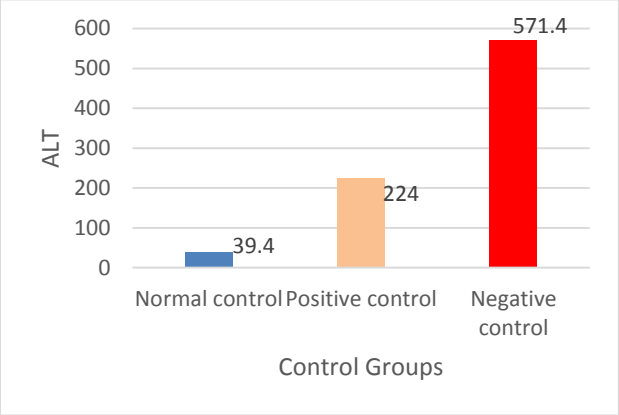

Figure1. Control groups for ALT

With Increasing dosesof Berberislycium extractit was seen in Group IV (Extract 1) , V (Extract 2) and VI (extract 3) the serum ALT alanine transferase levels were 232±42.3 U/L, 143±32.5 U/L and 62.2±18.2 U/L. respectfully (figure 2)

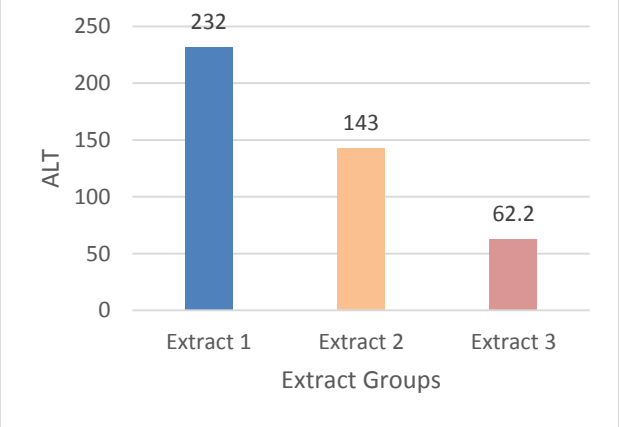

Figure 1. Experimental groups for ALT

Mean and SD in Group I serum ALP level was (Alkaline Phosphatase) was 247.6±29.1 IU/L Group II it was 146.8±75.9 IU/L and in group III it was571.4±123.3 U/L Figure 3

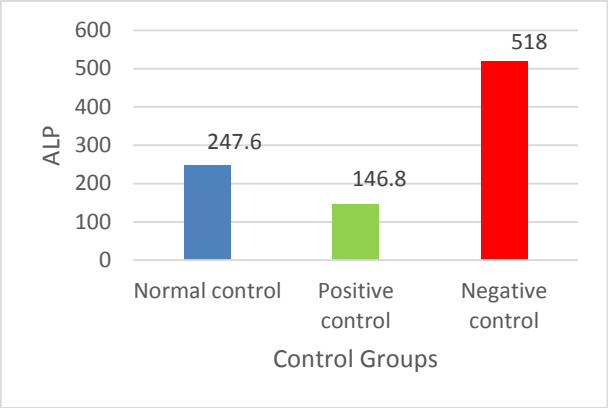

Figure 3. Control groups for ALP

In E1, group IV (100 mg/kg) ALP was 262±77.8 IU/L, in E2, group V (200 mg/kg) ALP was 184.6±46.8 IU/L and in E3, group VI (400 mg/kg) ALP was 155±34.4 IU/L.

In the figure (4) it is clearly shown that ALP kept on decreasing when we increase the dose of BerberisLycium. This further proved the hepatoprotective effect of BerberisLycium.

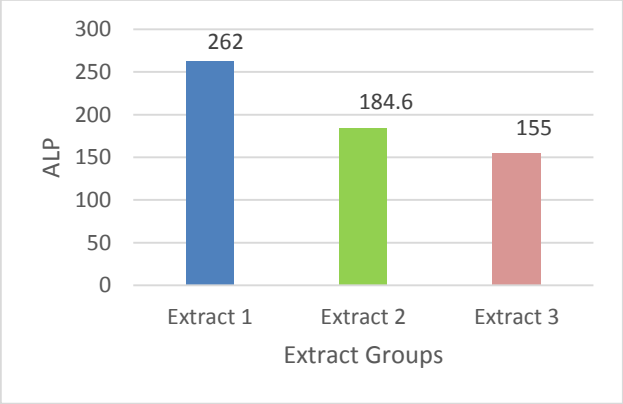

Figure 4. Experimental groups for ALP

DISCUSSION

Drug toxicity has been a matter of serious concern to medical practioner. 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 suchtoxicities.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.Mallhiet.al studied the hepatoprotective effect of Morusnigra in acetaminophen –induced liver toxicity.He suggested that herbal plants have greater potential to improve liver enzymes. [11]Literature review shows that many plant extracts have been studied to know about their hepatoprotective potential.Berberislycium 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 Berberislycium in liver ailments.

In present experimental study, acetaminophen-induced hepatotoxicity was obvious through biochemical test findings.Berberislycium decreased the elevated levels ofserum ALTlevels most significantly (p<0.000) and in the same way significantly reduced the raised serum ALP levels.

Manyresearch scholars have studied herbal plants for their antioxidant properties important for protecting various organs from oxidative damage.Rafiq et.al conducted a research using bark of Berberislycium 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.[12]

Girish et .al usedextracts of Berberislycium, Pistaciaintegerrima and Gallium aparine and found them beneficial in mice CCl_4-induced liver toxicity. [13]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. Their work supports our study for contributive hepatoprotective role of Berberis lyceum in combination of three herbs.

Berberislycium has shown hepatoprotective effect in another experimental study conducted by Purvikaet.al in rats which were given carbon tetra chloride. The ALT was reduced to normal after treatment with Berberis lycium.[14]The contradiction to our study is that in their study Purvikaused rats rather than mice as their experimental model. Furthermore he induced hepatotoxicity in rats by giving carbon tetra chloride solution and then Berberislycium was given.A similar study was conducted by Khan and his colleagues butthey used rats as their experimental model and observed beneficial results. [15] Antioxidant effect of Berberislycium were also proved UrRehman et.al. he said that hepatoprotective and antioxidant property of plant significantly decreased the raised serum ALT and ALP levels near to normal. [16]

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 Berberislycium in higher doses significantly (p<0.000) reversed the inflammatory changes in mice liver. Due to its edible nature, easy accessibility and economical factors Berberislycium 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 of the present study it is concluded that Berberislycium has hepatoprotective properties.The herbal plant has the ability to decrease the increased levels ofboth serum ALT and ALP levelsmost significantly. With increasing doses of the plant extract the liver functions

nearly came to normal.The studyresults support the traditional use of this herbal plant.

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