HEPATOPROTECTIVE EFFECTS OF BERBERIS LYCIUM, GALIUM APARINE AND PISTACIA INTEGERRIMA IN CARBON TETRACHLORIDE (CCL4)-TREATED RATS

Muhammad Aman Khan, Jehanzeb, Shafiullah, Salman A Malik, Muhammad Shafi

Department of Biological Sciences, Department of Pathology and Department of Agronomy
Quaid-i-Azam University, Islamabad,
Postgraduate Medical Institute, Lady Reading Hospital Peshawar and
NWFP Agricultural University, Peshawar - Pakistan

ABSTRACT

Objective: The aim of this study was to evaluate the hepatoprotective effects of mixture of berberis lycium, galium aparine and pistacia integerrima in carbon tetrachloride (CCL4)-treated rats.

Material and Methods: This study was conducted in the animal house of the Quaid-i-Azam University, Islamabad in the year 2000. A total of 20 rats were divided in 4 groups of 5 rats each.

1. Normal Control Group: with no medication given
2. CCl4 Treated Group: only CCl4 was given
3. Hepatopreventive group: Initially treated with medicinal plants mixture followed by CCl4
4. Hepatoprotective group: Initially treated with CCl4 followed by medicinal plants mixture

Then blood sample from each rate was collected and analysed for ALT, AST and ALP.

Results: The rates included in the study were male Sprague Dawley rats (albino) weighing 150-300g received from the animal house of the NIH, Islamabad. ALT , AST and ALP activities were significantly raised ($P<0.001$) in hepatocurative groups as comparative to the normal control, and decreased ($P<0.001$) as compared to the CCl4-treated rated and the hepatopreventive group. While the hepatocurative group treated with aqueous extract of the mixture of medicinal plants decrease more than treated with suspension of medicinal plants mixture and alcoholic extract.

Conclusion: The results this study indicates that a mixture of Berberis lycium, Galium aparine and Pistacia integerrima have hepatoprotective effects. These medicinal plants have more effect as curative agents rather than preventive agents.

Key words: Medicinal plants Berberis lycium, Galium aparine and Pistacia integerrima, hepatoprotective and hepatocurative role.

INTRODUCTION

The World Health Organization has defined traditional medicine as comprising therapeutic practices that have been in existence for hundreds of years[1]. The traditional preparations comprise medicinal plants, minerals and organic matter. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy[2].

Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India. More than fifteen of these plants are evaluated for their hepatoprotective action in light of modern medicine[3].

Many compounds known to be beneficial against carbon tetrachloride-mediated liver injury exert their protective action by toxin-mediated

DIFFERENT ENZYMES ACTIVITIES IN THE SERUM OF RATS TREATED WITH OLIVE OIL OR CCL4 WITH OR WITHOUT AQUEOUS SUSPENSION OF MEDICINAL PLANTS

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal Control group	036.40±01.21	027.40±02.02	179.2±25.31
CCl_4-treated group	198.60±23.82	361.80±52.84	582.4±22.02
Hepatopreventive group	105.80±04.32	310.00±33.28	571.0±28.18
hepatocurative group	079.40±06.19	109.20±06.55	225.8±08.06

Table 1

lipid peroxidation either via a decreased production of carbon tetrachloride derived free radicals or through the antioxidant activity of the protective agents themselves[4].

Berberis lycium: It is a prickly shrub locally known as "rasaunt" and belongs to the family Berberidaceae. It grows in Azad Kashmir, Baluchistan, N.W.F.P. and Punjab, at elevation of 900 – 2900m[5].

Galium aparine: Galium aparine is commonly called Prickly herb[5].

Pistacia integerrima: It belongs to the family Anacardiaceae. In Pushtu it is called "Shnaee". A middle-sized, deciduous tree with rough, grey bark grows in North Western Himalayas and Punjab[6].

Hepatoprotective action can be due to the flavonoids or the polyphenolic compounds, which are alcohol soluble and present in many plants. Flavonoids and polyphenolic compounds have potent antioxidant activity, which protect the liver against free radical injury[7]. Present study was designed as to evaluate the hepatoprotecttive effects of mixture of B. lycium, G. aparine and P. integerrima.

MATERIAL AND METHODS

Galium aparine whole plant was collected from wheat crop field in Nowshera, N.W.F.P, Pakistan. Bark of Pistacia integerrima was obtained from Swat, N.W.F.P., Pakistan. Bark of Berberis lycium was purchased from Khyber Bazaar Peshawar, N.W.F.P, Pakistan.

Preparation of powders and suspensions:

Bark of Berberis lycium, Whole plant of Galium aparine and bark of Pistacia integerrima were separately ground using mortar and pestle. The finally ground powder were stored in dry airtight containers. For preparation of suspensions, aliquots of powdered Galium aparine, Pistacia integerrima and Berberis lycium were mixed with distilled water (1, 1, 2 mg/ml respectively) and shaken vigorously for some time.

The aqueous suspension treatment was carried out by administering orally the desired material in the following dose rate:

Berberis lycium	150 mg/kg BW
Galium aparine	150 mg/kg BW
Pistacia integerrima	400 mg/kg BW

Animals:

Healthy male Sprague Dawley rats (albino) weighing between 150-300 g were obtained from the Animal House of National Institute of Health, Islamabad. They were then kept in the animal house at Quaid-i-Azam University, Islamabad. Food and fresh water was available ad libitum. A total of 20 rats were divided in 4 groups of 5 rats each.

1. Normal Control Group: with no medication given
2. CCl4 Treated Group: only CCl4 was given
3. Hepatopreventive group: Initially treated with medicinal plants mixture followed by CCl4
4. Hepatoprotective group: Initially treated with CCl4 followed by medicinal plants mixture

Induction of hepatic injury:

CCl_4 was used to induce hepatic injury with vehicle olive oil in 1:1 ratio (3 ml/kg body weight), which was given orally as single dose.

Collection of blood:

At sacrifice the animals were anaesthesized with diethyl ether and blood was collected by cardiac puncture using 5 ml syringe. The blood was transferred to test tube and allowed stand at room temperature for half an hour. The serum was separated from the clotted blood by centrifugation. The serum was then stored at -4°C in labeled eppendhrof tubes. The frozen sera were thawed and brought to room temperature before analysis.

Biochemical assays of blood:

Serum samples collected from control as

well as treated rats were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), using kits prepared by Randox Laboratories Ltd. Crumlin, UK. Instructions of the manufacturer were strictly followed during analysis. Measurements were made using Microlab 200 semiautomatic photometer of Merck.

Statistical Analysis:

All the data were expressed as mean ± SE. Student's t-test was applied for the difference between control and treated groups. $p<0.01$ was regarded as significant.

RESULTS

The results of the experiment to determine the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the serum of rats is given in the Table 1.

The table shows that in normal control rats, the serum activity of ALT, AST and ALP was only 36.4±1.21, 27.4±2.02 and 179.2±25.31 U/L respectively. The ALT in CCl_4-treated control raised more than five fold and became 198±23.82 U/L, AST raised more than thirteen times and became 361.8±52.84 U/L and ALP raised more than three fold and became 582.4±22.02 U/L. A similar and comparable increase in serum ALT, AST and ALP activities was also observed in the preventive groups where the activity in a mixture of Berberis lycium, Galium aparine and Pistacia integerrima treated rats was observed to be 105.8±4.32, 310.0±33.28 and 571±28.18 U/L respectively

However, the activities of serum ALT, AST and ALP in curative groups although significantly ($P<0.001$) increased as compared to the normal control remained considerably less both as compared to CCl_4-treated control as well as their respective counterpart in the preventive groups.

DISCUSSION

Toxin or drug-induced liver damage can mimic the effects of viral hepatitis [8]. Induced hepatic injuries are therefore commonly used as experimental models for the screening of CCl_4-induced hepatic injuries are therefore commonly used as experimental models for the screening of Hepato-protective drugs. The extent of the hepatic damage is often assessed by activities of cytoplasmic enzymes i.e. ALT and AST[9-11].

The present study was carried out to study the hepatoprotective effects of medicinal plants "Berberis lycium, Galium aparine and Pistacia integerrima" as these are locally used for the treatment of jaundice and liver inflammation by traditional healers.

In hepato-preventive study, medicinal plants treatment was given before the liver injury was induced to check if these prevent the damage to any extent where in hepato-curative study, medicinal plants treatment was given after the liver injury was induced to check if these cure the damage to any extent. CCl_4 was used for the induction of liver injury in our experiments. CCl_4 was given by 1.5 ml/kg body weight with vehicle olive oil in ratio of 1:1 in all CCl_4-treated group of rats while only olive oil was given by 1.5 ml/kg body weight in normal control group of rats.

There was a highly significant ($P<0.001$) increase in serum ALT and AST activities which increased respectively to about 5 and 8–13 times in rats treated with CCl_4, aqueous suspension of a mixture of Berberis lycium, Galium aparine and Pistacia integerrima in preventive as compared to normal control rats. There was a highly significant ($P<0.001$) increase in serum ALT and AST activities which increased respectively 2 and 4 times in rats treated with a mixture of Berberis lycium, Galium aparine and Pistacia integerrima in curative groups as compared to normal control rats. There was no significant change in serum ALT and AST activities in rats treated with aqueous suspension of a mixture of Berberis lycium, Galium aparine and Pistacia integerrima in preventive as compared to CCl_4-treated rats while there was a highly significant ($P<0.001$) decrease in serum ALT and AST in rats treated with aqueous suspension of a mixture of Berberis lycium, Galium aparine and Pistacia integerrima in curative group as compared to CCl_4-treated rats.

Previously it has been had reported that ALT and AST activities respectively increased by 5 –8 times and 10 – 13 times in CCl_4-treated rats as compared to the normal control rats[12]. So the present and the previous studies indicate that CCl_4 is such a hepatotoxin that induces sufficient injury in the liver of rats that can be detected from the transaminases i.e. ALT and AST released in the blood stream. Since ALT and AST activities weredecreased to half in rats treated with a mixture of Berberis lycium, Galium aparine and Pistacia integerrima in curative group as compared to other CCl_4-treated groups of rats therefore it appears that treatment of aqueous suspension of a mixture of Berberis lycium, Galium aparine and Pistacia integerrima as curative agents have the ability to protect the rats to some extent from CCl_4 damage of liver or cure to some extent the damage induced as a result of CCl_4 toxicity.

There was a highly significant (P<0.001) increase in serum ALP activity which increased about 3 times in rats treated with aqueous suspension of a mixture of Berberis lycium, Galium aparine and Pistacia integerrima in preventive group as compared to normal control rats. There was a highly significant (P<0.001) increase in serum ALP activity which increased about 1.5 times in rats treated with aqueous suspension of a mixture of Berberis lycium, Galium aparine and Pistacia integerrima in curative group as compared to normal control rats. There was no significant change in serum ALP activity in rats treated with aqueous suspension of a mixture of Berberis lycium, Galium aparine and Pistacia integerrima in preventive group as compared to CCl_4-treated rats. However, there was a significant (P<0.02) decrease in serum ALP activities in rats treated with aqueous suspension of a mixture of Berberis lycium, Galium aparine and Pistacia integerrima in curative group as compared to CCl_4-teated rats.

Wegwu MO et al[10], (2005) had given CCl_4 at the same ratio as used in our experiments (1.5ml/kg body weight). So the change in ALP activities was similar to our observations. ALP activity was decreased to half in Pistacia integerrima or a mixture of Berberis lycium, Galium aparine and Pistacia integerrima in curative group as compared to other CCl_4-treated rats which indicates that treatment of aqueous suspension of Pistacia integerrima or a mixture of Berberis lycium, Galium aparine and Pistacia integerrima as curative agents have the ability to protect the rats at least to some extent from CCl_4 damage of liver.

CONCLUSION:

The results of this study indicate that a mixture of Galium aparine, Berberis lycium and Pistacia integerrima have hepatoprotective effects. These medicinal plants have more effect as curative agents rather than preventive agents. The aqueous extracts of these medicinal plants are more effective in the treatment of hepatic injury as compared to alcoholic extract. The specific agent(s) in the extract responsible for the hepatoprotective effect, its characterization and mechanism of action needs to be further investigated.

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Address for Correspondence:
Muhammad Aman Khan
Department of Biological Sciences
Quaid-i-Azam University, Islamabad.
amankhank@yahoo.com