ORIGINAL ARTICLE

Hepatoprotective Effect of Tamarixdioica Roots on Acetaminophen Induced Hepatotoxicity in Male Mice

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ABSTRACT

Objective: To study the hepatoprotective effect of Tamarixdioica at low and high doses on transaminases (Alanine aminotransferase and Aspartate aminotransferase) in acetaminophen induced hepatotoxicity in male mice.

Study Design: It was a Randomized Control Trial.

Place and Duration of Study: The study was conducted at department of Pharmacology, Islamic International Medical College, Rawalpindi from 1st April 2015 to 31st March 2016.

Materials and Methods: Forty Balb-c Albino male mice were randomly divided in four groups with 10 mice each. Group A was the control group and received no medications. In Group B (Disease Control Group) hepatotoxicity was induced by Acetaminophen 1000mg per kg body weight given daily for 4 weeks. Group C (Low Dose Experimental Group) was given Acetaminophen 1000 mg/kg/day orally in combination with Tamarixdioica (Aqueous Extract) that was given daily through gavage tube in a dose of 100mg/kg/day for 4 weeks. Group D (High Dose Experimental Group) followed the same protocol as Group C but the Tamarixdioica (Aqueous Extract) dose was increased to 200mg/kg/day for 4 weeks through gavage tube. ALT and AST were measured and compared in different groups to see the hepatoprotective effect of Tamirixdioica.

Results: Mean ALT in Group A, B and C were 35.50 2.24 U/L, 94.00 8.62 U/L and 50.90 4.56 U/L respectively. There was a significant difference among ALT values of Group A & B and among Group B & C (p Value .000). Mean AST in Group A, B and C were 27.00 3.11 U/L, 151.00 14.53 U/L and 66.90 7.77 U/L respectively. There was a significant difference among AST values of Group A & B and among Group B & C (p Value .000). This suggested that hepatotoxicity was induced by acetaminophen and hepatotoxicity was improved by Tamirixdioica. No significant difference was observed in ALT & AST values among Group C and D.

Conclusion: Acetaminophen induces hepatotoxicity at high dose. Concomitant treatment with aqueous extract of roots of Tamarixdioica prevents hepatotoxicity induced by Acetaminophen in mice. Extract of roots of Tamarixdioica showed improvement biochemically in both low and high doses, as compared to drug treated group in a dose independent manner.

Key Words*: Acetaminophen, Hepatoprotective effect, Hepatotoxicity, Tamarixdioica.*

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Introduction

According to available data, diseases of liver such as inflammatory disorders and hepatitis are considered to be the most prevailing diseases in world. Pakistan has sixth highest number of cases with liver diseases.[1] Viral Hepatitis makes a big proportion in chronic cases.[2] Drug induced hepatotoxicity is common in Acute settings. Pharmacological drugs account for 20-40% of all cases of fulminant hepatic failure. Approximately 75% of the idiosyncratic drug reactions result in liver failure or death.[3] Hepatic injury caused by drugs is the most common reason mentioned for withdrawal of an approved drug. About 2000 cases of acute liver failure occur annually in the United States, and drugs account for over 50% of them (39% are due to acetaminophen, 13% are idiosyncratic reactions due to other medications).

Drugs account for 2-5% of total cases of patients hospitalized with jaundice and approximately 10% of all cases of acute hepatitis.[4] Acetaminophen (Paracetamol, N-acetyl-p-aminophenol) is the most commonly used over-the counter analgesic and antipyretic drug. At therapeutic doses, it is believed to be safe, having analgesic and antipyretic effects. Paracetamol toxicity is the foremost cause of acute liver failure in the Western world.[5] Estimation of liver enzymes should be done to evaluate the liver function during the course of hepatic injury treatment.[6] The sensitive markers for the evaluation of liver function are serum ALT and AST as these are cytoplasmic in their location and found in circulation in case of cellular damage. ALT is the most sensitive marker indicating the liver injury.[7]

Herbal medicines represent one of the most important fields of traditional medicine throughout the world. WHO estimates that 80% of the world population relies on herbal medicine for primary health care? About 30% of the pharmaceutical preparations are still extracted from plant material. These are the source of good affordable effective drugs. The family Tamaricaceae consists of 60 different species, which are also commonly called salt cedars. Tamirix.dioica (Tamaricaceae), with the local name of Lai in Urdu Ghaz or khagal, is an evergreen shrub or small tree with reddish bark, vaginate leaves, and purple flowers. The tree is native to Pakistan, Afghanistan, Iran, India, Bangladesh, Bhutan, Kashmir, Nepal, and Myanmar.[8] Screening of phytochemicals constituents of Tamarixdioica showed positive results for the presence of flavonoids, alkaloids, phenols, steroids, glycosides, carbohydrates, terpenoids and tannins. In past studies has been carried out which concluded the antioxidant and hepatoprotective effects of Tamarixdioica by using methanolic extracts of its various phytochemical.[9] However studies on hepatoprotective effects of Tamarixdioica by using aqueous extracts of its roots is limited. Objective of the present study was to observe the hepatoprotective effects of Tamarixdioica (Aqueous Extract) at low and high doses against acetaminophen induced hepatotoxicity in male mice by evaluating ALT and AST levels as biochemical markers.

Materials and Methods

This randomized control trial was conducted from 1st April 2015 to 31st March 2016 in the department of Pharmacology, Islamic International Medical College, Rawalpindi in collaboration with Animal house at National Institute of Health (NIH), Islamabad Pakistan after getting approval from Ethical Review Committee of Riphah International University (RIU), Islamabad.

In this study 40 adult male Albino mice were used. Mice weighing between 30-50 grams with normal Serum Alanine aminotransferase and Aspartate aminotransferase levels were included while mice weighing less than 30 grams or mice with abnormal serum ALT and AST were excluded from the study. Mice were first allowed to get acclimatized for one week in the NIH Animal house in 50-70% humidity at a room temperature of $24\pm2^{\circ}C$ with a 12-hour light and dark cycle.[8]

Blood samples were taken randomly from 8 mice for estimating Serum ALT and AST levels at day 0. Forty healthy mice were then randomly divided into four groups of ten mice each (n=10). Group A was normal control, given diet and water adlibitum for four weeks. Group B (Disease Control Group) was administered Acetaminophen at 1000 mg per Kg body weight through gavage tube daily for 4 weeks. Group C (Low Dose Experimental Group) was administered Acetaminophen 1000 mg/Kg/day weight along with Aqueous extract of Tamarixdioica at a dose of 100mg/kg/day through gavage tube for four weeks.[7] Group D (High Dose Experimental Group) was administered Acetaminophen 1000 mg/Kg/day weight along with Aqueous extract of Tamarixdioica at a dose of 200mg/kg/day through gavage tube for four weeks.

Roots of Tamarixdioica were purchased and authenticated from the plant sciences department, Quaid-e-Azam University, Islamabad. Aqueous extract of Tamarixdioica root was prepared at RIPS, Islamabad by using fine homogenized powder of Tamarixdioica which were mixed with distilled water, the whole solution was boiled for 2 hours and after cooling was filtered through filter paper what man no 3. The aqueous extract was formed by using vacuum rotary evaporator and was frozen dried. Sampling at day 0 was done via lateral tail vein. Sampling after four weeks was done through cardiac puncture. Biochemical analysis of serum ALT and AST was estimated through commercially available kits

by Merk and auto analyzer Microlab 300 on photometric system.

Statistical analysis of data was done by using SPSS version 21 and Mean ± Standard Error of Mean was calculated. One-way ANOVA and Post hoc Tuckey tests were applied to compare the mean difference between control and rest of the groups and mean difference in between the groups. P value of <0.05 was considered statistically significant.

Results

Initial serum ALT for eight randomly selected mice at day 0 was 33.21 ± 1.02 U/L whereas mean initial serum AST for eight randomly selected mice were 26.12 ± 1.60 U/L. There was no significant difference in the serum ALT and AST among these mice on day 0 (p Value .980).

Mean Serum ALT after four weeks for Group A (Normal Control Group) was 35.50 ± 2.24 U/L, for Group B (Disease Control Group) was 94.00 ± 8.62 U/L, for Group C (Low dose Experimental group) was 50.90 4.56 U/L & for Group D (High dose Experimental group) was 49.90 ±4.99 U/L. There was a significant difference in the values of Group A & B (p Value .000). This suggests that hepatotoxicity was induced by acetaminophen in group B. There was also significant difference in the values of Group B & C and in values of group B & D (p Value .000). This suggests that Hepatotoxicity in Group C & D was improved. More than Group D (Methanolic Extract Treated Group). There was no significant difference in the serum ALT levels among Group C & D Suggesting that both low and high dose of Tamarixdioica extract have an equal hepatoprotective effect. (P Value .999).

Mean Serum AST after four weeks for Group A (Normal Control Group) was 27.00 ± 3.11 U/L, for Group B (Disease Control Group) was 151.00 ± 14.53 U/L, for Group C (Low dose Experimental group) was 66.90 7.77U/L & for Group D (High dose Experimental group) was 53.00 ± 10.08 U/L. There was significant difference in the values of group A & B (p Value .000). This suggests that hepatotoxicity was induced by acetaminophen in group B. There was also significant difference in the values of group B & C and in values of group B & D (p Value .000). This suggests that Hepatotoxicity in Group C & D was improved. There was no significant difference in the serum AST levels among group C & D (p Value .748) Signifying that Aqueous extract of Tamarixdioica both in low and high dose have same efficacy in protecting liver against acetaminophen induced hepatic injury.

Table I: ANOVA of ALT and AST in all Groups after Four Weeks

		Sum of Squares	df	Mean Square	F	Sig.
ALT	Between Groups	19175.475	3	6391.825	20.421	.000
	Within Groups	11268.300	36	313.008		
	Total	30443.775	39			
AST	Between Groups	86285.075	3	28761.692	30.031	.000
	Within Groups	34478.900	36	957.747		
	Total	120763.975	39			

Table II: Multiple Comparison of Serum ALT and AST Among all Groups by Post Hock Tuckey Test

Parameter	Groups	Mean difference	P Value
Serum ALT	Group A (control group) Vs Group B (Disease Control Group)	-58.500(*)	.000
	Group A (control group) Vs Group C (Low Dose Experimental Group)	-15.400	.227
	Group A (control group) Vs Group D (High Dose Experimental Group)	-14.400	.281
	Group B (Disease Control Group) Vs Group C (Low Dose Experimental Group)	43.100(*)	.000
	Group B (Disease Control Group) Vs Group D (High Dose Experimental Group)	44.100(*)	.000
	Group C (Low Dose Experimental Group) Vs Group D (High Dose Experimental Group)	1.000	.999
Serum AST	Group A (control group) Vs Group B (Disease Control Group)	-124.000(*)	.000
	Group A (control group) Vs Group C (Low Dose Experimental Group)	-39.900(*)	.032
	Group A (control group) Vs Group D (High Dose Experimental Group)	-26.000(*)	.255
	Group B (Disease Control Group) Vs Group C (Low Dose Experimental Group)	84.100(*)	.000
	Group B (Disease Control Group) Vs Group D (High Dose Expe rimental Group)	98.000(*)	.000
	Group C (Low Dose Experimental Group) Vs Group D (High Dose Experimental Group)	13.900	.748

Discussion

Acetaminophen is the commonest analgesic and anti-pyretic used worldwide. Hepatotoxicity is produced by Acetaminophen and is deduced by the raised serum levels of ALT and AST. The current study investigates the protective effects of Tamarixdioica (Aqueous Extract) at low and high doses in Acetaminophen induced hepatotoxicity in male mice via biochemical parameters. We find that Acetaminophen induced hepatotoxic changes can be improved by both high and low doses of aqueous extracts of Tamarixdioica. In present study in comparison with the normal control Group A (which received normal standard diet) Acetaminophen induced hepatotoxicity is observed in Group B, Group C and Group D with resultant increase in levels of biochemical markers i.e. serum ALT and AST.

The present work is in accordance with findings of Boyd and Mitchell who reported hepatotoxicity in rodents when treated with higher doses of Acetaminophen.[11] whereas the rats were not very sensitive to the hepatotoxicity, both mice and hamsters proved to be more sensitive. Following this initial report, few cases of acetaminophen overdose were reported. Boyer and Rouff described the main clinical symptoms as development of nausea and vomiting, 2–3 h of ingestion followed by abdominal pain in the right upper quadrant. Liver dysfunction occurred within 24 h and reached a maximum approximately 3–4 days after ingestion.[12] The current study is also consistent with Prescott (Ihab Talat Abdel-Raheem; 2009) in which hepatotoxicity of Acetaminophen was evaluated by increased alanine aminotransferase (ALT) and aspartate aminotransferase levels along with mild hyperbilirubinemia, and increased prothrombin time.[13]

In our study when results of Group C (Low Dose Experimental Group) and Group D (High Dose Experimental Group) were compared regarding ALT and AST no significant difference was observed (p Value > 0.05). These results indicate that hepatoprotective role of Tamarixdioica remain almost same when given in low or high Dose and it is not dose dependent. Similar findings were observed by Krishnaiah and his colleagues in which they found the hepatoprotective role of Tamarixdioica in both low and high doses. They have attributed this hepatoprotective property of Tamarixdioica to presence of flavonoids and tannins present in abundance in Tamarixdioica.[6]

Our study is also in accordance with study carried by Abouzid S and his colleagues which showed a marked reduction in tissue glutathione level in rats. The hydro-alcoholic extract of Tamarix (100 mg/kg body weight) ameliorated the adverse effects of carbon tetrachloride on Hepatocytes and returned the altered levels of biochemical markers near to the normal levels. We have compared results of Tamarixdioica to acetaminophen and not CCl4.[14] However same mechanism has probably produced hepatoprotective effect in our study also.

Previously studies have been done on exploring hepatoprotective effect of Tamarixdioica in combination with medical and other herbal compounds and extracts. No dose dependent study was done individually on aqueous extract of Tamarixdioica roots extract which guides us about the submaximal, ceiling effect and toxicity. Our study confirms the hepatoprotective effect of aqueous extract of Tamarixdioica roots both in low and high dose. Further studies are needed to determine dosage for submaximal and ceiling effect of Tamarixdioica roots extract. In addition a different route of administration can be tried to see the same effect.

Conclusion

Aqueous extract of Tamarixdioica roots have significant hepatoprotective effect on Acetaminophen induced hepatotoxicity both in low and high doses.

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