Original Article

Protective Effects Of Tecomella Undulata Stem Bark Extract On Isoniazid Induced Hepatotoxicity: Based On Liver Enzymes And Histopathology In Rat Model

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ABSTRACT:

Objective: To evaluate the hepatoprotective effects of Tecomellaundulata stem bark extract on isoniazid induced hepatic damage based on liver enzymes and Liver function test in rat models.

Study design and Setting: An experimental study conducted at Department of Pharmacology at Al-Tibri Medical College and Hospital, Isra University Karachi Campus and Dow University of Health Sciences, Karachi.

Methodology: Total twenty-four rats were studied. The albino rats that were male, healthy, and weighing 200-250grams were included in this study. Rats were divided into four groups, each group having six rats and treated once daily orally for 30 days. Group A was control group and treated with normal animal diet and water; Group B was Isoniazid treated group and induced by oral administration of Isoniazid (INH) 50mg/kg. Group C was treated with Isoniazid 50mg/kg and Tecomellaundulata bark extract with low dose of 200mg/kg . Group D was treated with Isoniazid 50mg/kg and Tecomellaundulata bark extract with high dose of 400mg/kg . All the animals were weighed before commencement of the study. Liver enzymes were noted after the end of experiment. P value of <0.05 was taken as significant.

Results: While comparing the mean values of AST,ALT, ALP and GGT in all four groups group; the statistical significant difference ($p<0.001$) was found. The mean levels in of total Bilirubin in group A was 0.69 ±0.01, group B 1.04 ±0.04, in group C was 1.15 ±0.39, and in group D was 1.04 ±0.44 with the significant difference ($p=0.004$).

Conclusion: Tecomellaundulata has a protective effect on isoniazid induced toxicity on liver as evidenced by liver function test on rat models.

Keywords: Isoniazid induced hepatotoxicity, Liver enzymes, Tecomellaundulata

INTRODUCTION:

The plants are used as medicine due to low cost and natural source of remedy. According to WHO, 80% of developing countries are using medicinal plants for the sake of treatment.[1-3] It is a well-established fact that there are numerous plants used to derive hepato-protective medicines as they contain various kinds of phytoconstituents like polysaccharides, proteins, flavonoids, lignans, and rotenoids etc which enhanced the immune system and are involved in treating different hepatic diseases. The herbal plants are less expensive and have fewer side effects.[4] The mechanisms used to protect liver are degeneration of free radicals by increasing antioxidants.[5,6] Tecomellaundulata is a member of family Bignoniaceae. It is a flower grown on a small tree mostly in Saudi Arabia, Northwest of India and Southern Pakistan.[7] Locally it has given various names like Roheda, Lohira, Rohira and Purpak in different regions of Pakistan. In Pakistan it is found in Khuzdar, Baluchistan.[8-10] Whole plant including bark, seeds, roots, and flowers are used in treatment of various ailments. The flower is mostly used for treatment of hepatitis and seeds for abscess treatment.[11-13] Flowers of this plant are rich in Quercetin, Rutin and â- sitosterol.[14-16] The liver has various functions in body like metabolism of drugs, removing toxic material from body, vitamin storage, protein formation and lipid synthesis.[17] The liver can be damaged by hepatocellular injury, cholestatic damage and mixed injury in which alkaline phosphatase (ALP) is increased. The risk factors for liver injury are alcohol consumption, smoking, genetic and drug toxicities.[18] Isoniazid is the first line of therapy for treatment of tuberculosis. It is a prodrug with oral dosage of 10-15 mg/kg/day in children while, in adults it is 300mg/day.[19] Although the toxic dose in rats is 200 mg/kg body weight.[20] Isoniazid is an inactive drug that is activated by enzyme catalase peroxidase. The

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Received: 20-11-2019
Accepted: 13-12-2019

bacterial catalase peroxidase enzyme is secreted by mycobacterium tuberculosis. The active form of drug reduces nicotinamide adenine dinucleotide (NADH). This inhibits formation of mycolic acid in mycobacterium, which is a pivotal cell wall component.[21] The oral absorption of drug is rapid, and it diffuses well in all body fluids and tissues. According to authors knowledge; there is no literature available to treat the liver toxicity with herbal option in Pakistan and indeed it was the rationale of the study. Therefore, this study was aimed to evaluate the levels of liver enzymes and LFTs in rat models receiving Tecomellaundulata stem bark extract on liver toxicity induced by isoniazid and to evaluate the histopathology of liver after using Tecomellaundulata on isoniazid induced liver toxicity rats.

METHODOLOGY:

It was an experimental study; carried out in the Department of Pharmacology at Al-Tibri Medical College and Hospital, Isra University Karachi Campus and Dow University of Health Sciences, Karachi. The duration of the study was seven months from May 2018 till November 2018.This study had adult male albino wistar strain rats as experimental model selected from the animal house of Al-Tibri Medical College and Hospital Karachi. All animals were kept under standard laboratory conditions at temperature 27±2 °C and light and dark cycle 12/12 hour. The animals were fed on standard laboratory feed and water. Total twenty-four rats were studied. The rats that were male, healthy, albino, weighting 200-250grams were included in this study. The rats that were female, unhealthy or less than 200grams in weight were excluded from this study. The rats were divided into four groups having six rats in each group. These groups were A, B, C and D. the group A was control group, it had rats that were treated with normal animal diet and water orally for 30 days. The group B was Isoniazid treated group, in this group the hepatic injury was induced by oral administration of Isoniazid (INH) 50mg/kg once daily for 30 days. The group C was Isoniazid 50mg/kg and Tecomellaundulata bark extract with low dose, in this group Isoniazid 50mg/kg and Tecomellaundulata stem bark extract 200mg/kg were given orally once daily for 30 days. The group D was Isoniazid 50mg/kg and Tecomellaundulata bark extract with high dose, in this group Isoniazid 50mg/kg along with Tecomellaundulata stem bark extract i.e., 400mg/kg once daily per oral for 30 days. All the animals were weighed before commencement of the study. The animals were kept in separate cages. Two rats in each cage. The animals were given free access to diet and water and libitum. The animals were acclimatized to expose 12-hour light and 12-hour dark circadian cycle. The animal's condition and health were accessed by the gain or loss of weight and weakness of animals. After 30 days the animals in these groups were sacrificed under anesthesia. Liver was exposed and preserved for histopathology and blood 3ml was drawn by cardiac puncture for biochemical parameters (liver enzymes) like Alkaline phosphates (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT)and bilirubin. The data was recorded on excel spread sheets and analyzed on SPSS version 20. Means and standard deviation (SD) were calculated for quantitative data and Kruskal Wallis Test was applied to compare the values among four groups and p value = 0.05 was considered as significant.

RESULTS:

While comparing the mean values of AST, ALT, ALP and GGT in all four groups group; the statistical significant difference ($p<0.001$) was found. The mean levels in of total Bilirubin in group A was 0.69 ±0.01, group B 1.04 ±0.04, in group C was 1.15 ±0.39, and in group D was 1.04 ±0.44 with the significant difference ($p=0.004$).The mean levels in of direct Bilirubin in group A 0.02 ±0.01, group B 0.75 ±0.04, in group C was 0.80 ±0.07, and in group D was 0.47 ±0.23 with the significant difference ($p<0.001$). (Table: I)

DISCUSSION:

In our study the plant Tecomellaundulata (RohitakaGhrita) showed a protective effect on isoniazid induced liver injury on rat models. There was a protective effect of this herb on liver in paracetamol induced liver injury.[22] This plant was suggested to cure liver diseases at dose of 3.6gm/kg in a study.[23] In a recent study there was a hepatoprotective effect of plant Tecomellaundulata against liver injury.[24] It is a well known way to produce liver damage in rats by medicines;[25] there is marked increase in SGPT and SGOT levels that are released into systemic circulation due to hepatic cellular membrane damage. However, Tecomellaundulata reduces these levels markedly. The intoxication facilitates the release of alkaline phosphatase due to hepatic damage and it was remarkably reversed by Tecomellaundulata. Therefore, the decline in level of enzymes indicate membrane stabilizing role of Tecomellaundulata. In addition, the increase in serum bilirubin levels is a predictable way of assessing liver damage which was markedly decreased by Tecomellaundulata showing its efficacy as hepato protective agent. The free radical generation is thought to cause lipid peroxidation, depletion of glutathione and catalase[26] which is consistent with our study in which we also observed the protective effect. However, the study states that treatment with Tecomellaundulata can markedly restore the defense mechanism by increasing glutathione, catalase and hence decreasing peroxidation of cellular membrane. The results of our study outcomes were congruent with the another study in which prominent improvement was noted on histopathology of rats receiving Tecomellaundulata in toxicity induced by paracetamol, there was a hepatoprotective effect of Tecomellaundulata on hepatic tissue in other study.[22] Considering the views of our study and to what extent the levels of enzymes in these rats are associated with the

Table I: Levels of liver enzymes in various groups of Albino Rats

	Groups	Mean ± SD	Median (IQR)	p-value
AST	**A**	127.61 ± 1.94	127.39(125.86 – 129.52)	<0.001
	B	722.86 ±2.54	723.04(720.30 – 724.96)	
	C	350.46 ±1.59	349.99(349.06 – 352.13)	
	D	160.46 ±1.03	160.41(159.73 – 161.25)	
ALT	**A**	35.82 ± 2.03	35.51 (34.43 – 37.55)	<0.001
	B	91.59 ± 1.61	91.46 (89.96 – 93.11)	
	C	80.76 ± 1.95	80.40 (78.96 – 82.44)	
	D	49.39 ±1.42	49.74 (48.42 – 50.30)	
ALP	**A**	130.60 ± 1.21	130.48 (129.63 – 131.87)	<0.001
	B	271.18 ± 1.66	271.04 (269.70 – 273.02)	
	C	216.26 ± 2.08	216.46 (214.52 – 218.13)	
	D	178.91 ± 2.24	179.51 (176.61 – 180.97)	
GGT	**A**	8.95 ± 0.34	9.00 (8.80 – 9.14)	<0.001
	B	38.05 ± 2.21	38.01 (35.88 – 40.25)	
	C	29.43 ± 1.70	29.29 (28.32 – 31.08)	
	D	14.76 ± 1.23	14.96 (13.71 – 15.45)	
Total Bilirubin	**A**	0.69 ±0.01	0.69 (0.67 – 0.71)	0.004
	B	1.04 ±0.04	1.06 (0.98 – 1.06)	
	C	1.15 ±0.39	1.00 (0.98 – 1.25)	
	D	1.04 ±0.44	1.06 (0.98 – 1.06)	
Bilirubin Direct	**A**	0.02 ±0.01	0.02 (0.02 – 0.04)	<0.001
	B	0.75 ±0.04	0.75 (0.71 – 0.79)	
	C	0.80 ±0.07	0.83 (0.77 – 0.85)	
	D	0.47 ±0.23	0.47 (0.44 – 0.49)	

histological findings of the liver will be useful to discover more facts about the plant. The limitations of our study included measurement biases or observer bias and was conducted on small scale and hence results cannot be projected on general population. It is recommended that Tecomellaundulata is medicinally and economically important tree of arid regions of India and Pakistan. Due to its increasing demand in timber and pharmacological industry it is becoming extinct. The tree is grown slowly through seeds and there is no alternate method for its quick breeding. There is great need to preserve, reproduce and protect it so its beneficial medicinal uses can be sustained and available for prompt treatment of liver toxicity.

CONCLUSION:

Tecomellaundulata has a protective effect on isoniazid induced toxicity on liver as evidenced by liver function test on rat models. It was also predicted that in liver-injury induced rats, Tecomellaundulata can reverse the toxicity caused by isoniazid to the liver and thereby improving the overall status of liver enzymes.

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