Original Article

Effect of Terminalia Arjuna (Arjun) Bark on Serum Aspartate Aminotransferase and Alanine Aminotransferase in Isoniazid Induced Hepatotoxicity in Wistar Albino Male Rats

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Abstract

Background: *Liver diseases have become a major problem worldwide and are associated with significant morbidity and mortality. Many of the new drugs used for the treatment for liver diseases. They are costly and have limited efficacy. Therefore, plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seem to be helpful for treating liver diseases. The Objective of the study is to observe the hepatoprotective effect of Terminalia arjuna on serum aspartate aminotransferase and serum alanine aminotransferase in isoniazid induced hepatotoxicity in Wistar albino male rats.* ***Materials and Methods:*** *This experimental study was carried out in the department of physiology, Dhaka Medical College, Dhaka from July 2021 to June 2022. A total number of twenty-four (24) apparently healthy Wistar albino male rats (age 90-120 days) weighing between 150-200 gm were selected for the study. After acclimatization for 7 days, they were divided into control groups and experimental groups. Control group was subdivided into group A (baseline control group) and group B (isoniazid only treated group). The experimental group was subdivided into group C (Isoniazid pretreated and Terminalia arjuna treated group) and group D (Isoniazid pretreated and Terminalia arjuna treated group). On day 22, all the animals were sacrificed, and blood samples were collected from the heart. The liver samples were excised preserved in 10% formalin for histology. Serum AST and ALT were estimated. Data were presented as mean ±SD. One way ANOVA or Kruskal Walli's test followed by Bonferroni test was done.* ***Results:*** *The serum ALT and AST level were significantly increased in group B (135.3±8.1, 119.5±8.5) in comparison with that of group A (42.8±5.8, 40.5±9.4); group C (58.1±19.9, 54.1±13.9) and group D (55.5±14.1, 46.1±12.7). No significant difference was found in serum ALT and AST levels between group A and group C; group A and group D and between group C and group D.* ***Conclusion****: The results of this study may be suggested that Terminalia arjuna has hepatoprotective effect on Isoniazid induced liver damage in Wistar albino male rats.*

Key words: *Terminalia arjuna*, Hepatoprotective, Isoniazid, Wistar Rats.
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Introduction

The liver is the major metabolic organ and largest gland of the human body which plays a central role in different types of nutrients metabolism. It is also concerned with various types of essential functions such as synthesis of proteins, synthesis of coagulation factors, storage of vitamins and minerals, formation and secretion of bile, detoxification of drugs and toxic metabolites and takes part in immune regulation of the body[1]. Liver disease is now considered as one of the leading health problems globally. Millions of people are affected by liver disease worldwide and approximately 2 million dies yearly[2]. It has become the highest disease burden in developing countries due to largely neglected issue[3]. In Bangladesh, liver disease including both acute and chronic viral hepatitis with jaundice, Non-Alcoholic Fatty Liver Disease as well as chronic liver disease has become one of the major treatment burdens. The numbers of patients with NAFLD, a noncommunicable disease, are increasing day by day too[4].

Drug induced liver injury (DILI) or hepatotoxicity is an adverse drug reaction occurs by exposure to

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drugs. DILI can be categorized as either predictable or unpredictable (idiosyncrasy). The incidence of predictable DILI is much higher than the unpredictable DILI[5]. Unpredictable or idiosyncrasy DILI can be explained as unexpected adverse hepatic reaction based on the pharmacological action of drug where predictable DILI usually occurs as a result of drug overdose[6]. Estimation of transaminase and bilirubin level are helpful in diagnosis of toxicity of liver[7]. Some specific laboratory criteria are used to identify the DILI. Usually, 3-5 times elevation of liver enzymes or in case of bilirubin, greater than upper limit of normal range is required[8].

More than a thousand drugs and chemicals have been implicated in drug induced toxicity of liver[9]. Antibiotics, Anti-epileptics, herbal medicines and Non-Steroidal Anti-Inflammatory Drugs (NSAID) are the major etiologic agents for drug induced hepatotoxicity. Although there are regional variations and limited data on etiology of DILI in developing countries, anti-tuberculosis drugs have been found the leading cause of drug induced hepatotoxicity[10]. Tuberculosis (TB) is a curable disease that requires long term treatment duration with multiple drugs[11]. Most common regimen for the treatment of TB uses the four first line drugs isoniazid, rifampin, pyrazinamide and ethambutol for the first two months of treatment followed by four months of Isoniazid and Rifampicin[12]. Isoniazid is an antibiotic used as part of the initial treatment regimen for Mycobacterium tuberculosis infection. It kills M. tuberculosis by inhibiting bacterial cell wall synthesis[13]. The manifestations of drug induced toxicity of hepatocytes are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure[14].

Isoniazid can also produce similar liver lesions in experimental animals as toxic reaction in liver tissue is one of the most common side effects of anti-tuberculosis regimens[15]. About 10 to 20% of patients taking isoniazid have a transient rise of serum ALT level (>10 times upper limit of normal). The prevalence of Antituberculosis treatment (ATT) induced hepatotoxicity is 11.6% in India compared to western countries where the prevalence is 4.3%[16]. Medicinal plants play a major role in primary health care as they are essential for preparing the raw materials for both conventional and traditional medicine. They earned their popularity due to effectiveness, easy availability, lack of current medical alternatives, higher cost of modern medicine and cultural and social preference[17]. *Terminalia arjuna* is a traditional plant medicine found in tropical and subtropical regions famous for its medicinal properties. In Bangladesh it is locally known as Arjun which is a member of Combretaceae family[18]. It consists of many phytoconstituents including arjunic acid, flavonoids, saponins, tannins, phytosterol, glycosides, alkaloids etc. These components of *Terminalia arjuna* have been shown to their effectiveness in antioxidant and anti-inflammatory activities in acute liver injury[19].

In view of the medicinal values of traditional herbal plants, the present study has been designed to observe and establish the hepatoprotective effects of *Terminalia arjuna* against Isoniazid induced hepatotoxicity in adult rats.

Materials and Methods

The Experimental study (animal model) was carried out in the Department of Physiology, Dhaka Medical College, Dhaka from July 2021 to June 2022. A total number of 24 apparently healthy Wistar albino male rats (age ranging from 90 to 120 days) and weighing 150 to 200 gm were selected purposively for this study. Female and Unhealthy rats, age less than 90 days and more than 120 days and weight less than 150 gm were excluded from the study. Ethical approval was obtained from the ethical review committee of Dhaka Medical College (ref: ERC-DMC/ECC/2022/72). The animals were purchased from the animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka and Preparation of extract of *Terminalia arjuna* bark was done at the Institute of Nutrition and Food Science, Dhaka University. The rats were kept in metallic case in the animal house of Institute of Nutrition and Food Science, University of Dhaka. They were kept in a standard laboratory condition on 12/12 hours light/ dark cycle for 7 days acclimatization prior 21 days of experiment[20]. Throughout the acclimatization and experimental period, the rats had unlimited access to food and distilled water.

After acclimatization for 7 days, all the animals were divided into group A, group B, group C and group D based on the treatment. After grouping, the initial body weight of all the rats were measured on day 1.

i. Group A (baseline control group, n=6): They received basal diet for 21 consecutive days. In addition to basal diet, they received normal saline orally (1 ml/kg body weight/day) for 21 days.

ii. Group B (isoniazid treated control group, n=6): They received basal diet for 21 consecutive days. In addition to basal diet, they received tablet isoniazid orally (100 mg/kg body weight/day) for 21 consecutive days.

iii. Group C (Isoniazid pretreated and aqueous extract of *Terminalia arjuna* bark treated group, n=6): They received tablet isoniazid orally (100 mg/kg body weight/day) for consecutive 21 days and aqueous extract of *Terminalia arjuna* bark (250 mg/kg body weight/day, orally) simultaneously for 21 consecutive days.

iv. Group D (Isoniazid pretreated and aqueous extract of *Terminalia arjuna* bark treated group, n=6): They received tablet isoniazid orally (100 mg/kg body weight/day) for consecutive 21 days and aqueous extract of *Terminalia arjuna* bark (500 mg/kg body weight/day, orally) simultaneously for 21 consecutive days.

On day 22, the final body weights of all the rats were recorded before the sacrifice. After all the rats were anaesthetized with 30% chloroform, blood sample (approximately 4 ml) was collected from the heart in separate clean and dry test tubes with proper identification number from each rat. Then blood was centrifuged at a rate of 4000 rpm for 5 minutes. After separation, serum was collected in a labelled Eppendorf's tube and preserved in a refrigerator for biochemical (serum ALT and AST) analysis.

All the parameters were expressed as mean ±SD (standard deviation) and percentage. One way ANOVA test or Kruskal Walli's test was done to compare all the parameters among the groups followed by Bonferroni test to compare between the groups. *p* value of <0.05 was considered as level of significance. Statistical analysis was performed by using a computer based statistical program SPSS Version 23.

Results

In this study the mean±SD initial body weights of the rats were 164.5±6.6, 161.3±5.5, 166.8±9.9 and 168.1±7.1 gm on day-1 followed by final body weight were 169.1±6.0, 166.1±6.0, 170.6±9.9, 171.8±8.5 gm on day-22 in group A, group B, group C and group D respectively. The mean±SD of both initial and final body weight of the rats in group B was lower in comparison to group A, group C and group D but no statistically significant difference was found among them. Figure-1 showed the mean % change of body weight in four (4) groups of rats.

The mean±SD ALT levels of the rats were 42.8±5.8, 135.3±8.1, 58.1±19.9 and 55.5±14.1 U/L & the mean±SD S. AST levels were 40.5±9.4, 119.5±8.5, 54.1±13.9 and 46.1±12.7 U/L in group A, group B, group C and group D respectively. In this study, mean±SD serum level of ALT and AST was higher in group B in comparison to that of group A and the difference was found statistically significant (p<0.05). Again, it is also higher in group C and group D comparison to group A, but the difference was not statistically significant (p>0.05). Furthermore, the level was significantly lower in group C and group D in comparison to that of group B. But the mean±SD level of serum ALT & AST had no significant difference between group C and group D.

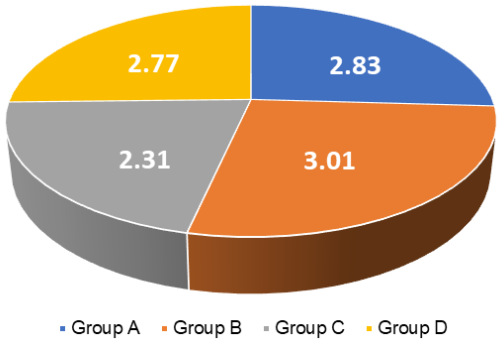

Figure-1: Pie chart shows percent (%) change of body weight in different groups of rats (n=24)

Table-I: Initial and final body weight in different groups of rats (n=24)

Parameters	Group A (n=6)	Group B (n=6)	Group C (n=6)	Group D (n=6)	Groups Comparison	p-value
Initial Body Weight (I) (gm)	164.5±6.6	161.3±5.5	166.8±9.9	168.1±7.1	A vs B vs C vs D A vs B A vs C A vs D	0.43^{ns} 1.00^{ns} 1.00^{ns} 1.00^{ns}
Final Body Weight (F) (gm)	169.1±6.0	166.1±6.0	170.6±9.9	171.8±8.5	B vs C B vs D C vs D	1.01^{ns} 0.77^{ns} 1.00^{ns}

ns=non-significant

Table-II: Serum ALT in different groups of rats (n=24)

Parameter	Group A (n=6)	Group B (n=6)	Group C (n=6)	Group D (n=6)	Groups Comparison	p-value
Serum ALT (U/L)	42.8±5.8	135.3±8.1	58.1±19.9	55.5±14.1	A vs B vs C vs D A vs B A vs C A vs D B vs C B vs D C vs D	$<0.001^{S}$ $<0.001^{S}$ 0.34^{ns} 0.67^{ns} $<0.001^{S}$ $<0.001^{S}$ 1.00^{ns}

s=significant; ns=non-significant

Table-III: Serum AST in different groups of rats (n=24)

Parameter	Group A (n=6)	Group B (n=6)	Group C (n=6)	Group D (n=6)	Groups Comparison	p-value
Serum AST (U/L)	40.5±9.4	119.5±8.5	54.1±13.9	46.1±12.7	A vs B vs C vs D A vs B A vs C A vs D B vs C B vs D C vs D	<0.001^{S} <0.001^{S} 0.30^{ns} 1.00^{ns} <0.001^{S} <0.001^{S} 1.00^{ns}

s=significant; ns=non-significant

Discussion

This study was conducted to investigate the hepatoprotective effects of Terminalia arjuna against isoniazid-induced liver damage in rats. A total number of twenty-four (24) Wistar albino rats, weighing 150 to 200 gm, age ranging from 90 to120 days were used in the present study. Then the rats were divided into 4 groups. After grouping, the initial body weight of all the rats were measured on day 1. Then all rats were sacrificed on day 22. Prior to sacrifice, the final body weights of all the rats were recorded.

In the present study, initial (I) and the final (F) body weight of all rats were almost similar and showed no statistically significant difference. The mean % change of body weight was almost similar, and the difference was not statistically significant between baseline control and experimental group. The liver enzymes (ALT and AST) were significantly higher in the isoniazid control group in comparison to that of baseline control group and experimental groups. Elevated levels of hepatic enzymes in liver damage can be normalized with healing of parenchyma and regeneration of hepatocytes by treatment with bark extract of *Terminalia arjuna.* This is probably because of the free radical scavenging properties of flavonoids and polyphenols. The presence of highest content of phenolic acid in *Terminalia arjuna* extract is also responsible for improving liver function[20].

Extract of *Terminalia arjuna* facilitates the rapid & efficient consumption of reactive oxygen species produced by isoniazid P_{450} bioactivation. This medicinal plant attenuates the production of ROS by inhibiting IL-1β and thus prevents the secondary cell mediated death. It also inhibits caspase-9, a cysteine protease responsible for execution of apoptosis, and thus prevents abnormal apoptotic pathway of cell death[19]. Extract of *Terminalia arjuna* bark elevates the glutathione level of the hepatocytes that bind with drug metabolites and finally reduces the toxic effects of isoniazid metabolites[21].

Conclusion

In this study serum ALT and AST levels were significantly increased only in the isoniazid treated group of rats. Again, serum ALT and ALT levels were significantly decreased in isoniazid pretreated and *Terminalia arjuna* treated group of rats. So, it might be suggested that *Terminalia arjuna* has hepatoprotective effect on Isoniazid induced liver damage in Wistar albino male rats.

Conflict of interest

The authors declared that they have no conflict of interest.

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