Effect of Terminalia Chebula (Haritaki) Extract on Serum Liver Marker Enzyme (ALP) in Paracetamol Induced Liver Damage in Wister Albino Rats

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Medicinal plants play a key role for healthy living of an individual. Liver is a vital organ which plays major role in metabolism and excretion of toxic substances from the body. Liver diseases remain one of the serious health problems. Harbal plants as Terminalia chebula (Haritaki) may have free radical scavenging activity thereby can be used for the prevention and treatment of liver damage. To observe the effect of Terminalia chebula extract on paracetamol induced change of serum liver marker enzyme eg Alkaline Phosphatase (ALP) level in Wister albino rats. This experimental study was carried out in the Department of Physiology, Dhaka Medical College, Dhaka from Jan' 2013 to Dec' 2013. A total number of 44 rats, age ranging from 90 to 120 days, weight between 150 to 200 gm (initial body weight) were selected for the study. After acclimatization for 14 days, they were divided into control groups (n=22) and experimental groups (n=22). Control groups were subdivided into base line control (BC, n=11) and paracetamol treated control (PC, n=11). Experimental groups were again subdivided into Terminalia chebula pretreated and paracetamol treated (TCP-PCT, n=11) and paracetamol pretreated and Terminalia chebula treated group (PCP-TCT, n=11). Before sacrifice, final body weights of all the rats were measured. Then all the rats were sacrificed on 22nd day and then blood samples were collected. For assessment of liver function, serum ALP level was done by using standard laboratory kits. The statistical analysis was done by one way ANOVA and Bonferroni test as applicable. The mean serum ALP level was significantly ($p<0.001$) higher in paracetamol treated control group in comparison to those of baseline control group. Serum ALP level of all experimental groups were significantly ($P<0.001$) lower than PC group. Terminalia chebula extract restored serum ALP towards the normal level in paracetamol induced liver damage in rats which may be due to its free radical scavenging activity.

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Key words: Terminalia chebula, Hepatoprotective, Paracetamol, Alkaline phosphatase

Introduction

Herbal medicines have great demand in developed world for primary health care because of their less side effect. About 80% population of the world rely on traditional medicine which is predominantly based on plant materials.[1] Terminalia chebula had been used in folk medicine throughout the ancient times in Bangladesh. Locally it is known as Haritaki and a member of Combretaceae family. It is also called the king of medicine because it has been widely used in ayurveda, unani, siddha and homeopathy.[2] It is reasonably cheap, available and safe. The phytochemical investigation of T. chebula shows the presence of several phenols such as gallic acid, ellagic acid, tannic acid, ascorbic acid, ß-sitosterol, ethyl gallate, chebulic acid, and mannitol. Different researchers from different countries have studied the hepatoprotective effects of Terminalia chebula due to their active ingredients.[3]

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Liver is one of the most important organ in the human body. It plays an important role in detoxification and excretion of many endogenous and exogenous compounds.[4] It is continuously and widely exposed to toxin and different types of exogenous compounds that lead to impairment of its function.[5] Liver diseases are major health problems worldwide, with high endemicity in developing countries.[6] Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorder. Some synthetic drugs like analgesic, antimicrobial, NSAIDs etc are currently used in the treatment of liver diseases have been reported to cause serious adverse side effects.[7] Paracetamol is an analgesic and antipyretic drug which is widely used to cure headache, fever, and other pains and is readily available without prescription. Increasing use and easy available of paracetamol have led to misuse of the drug and may cause a number of serious clinical problems.[8] Paracetamol is hepatotoxic when used in excessive doses or when used in therapeutic doses for a prolonged period.[9,10]

It has been reported that medicinal plants have less or no side effect. In medicinal practices, reliable liver protective drugs are not available but herbs may play an important role in management of liver disorders.[11] Recently, some investigators observed that Terminalia chebula significantly decreased the paracetamol induced elevation of serum ALP in rats.[12,13] Now a days there is increasing need for substances to protect the liver from damage. Modern medicines have more side effects at doses required to cure liver diseases but remedies by medicinal plants have lesser side effects for the treatment of liver diseases. Therefore, the present study has been designed to examine the hepatoprotective effect of Terminalia chebula extract on paracetamol induced liver damage in Wister albino rats. It is also expected that the result of this study would make the Terminalia chebula extract acceptable among the people as a rich source with medicinal value for the prevention of liver damage.

Methods

This experimental study was conducted from January 2013 to December 2013 in the Department of Physiology, Dhaka medical college (DMC), Dhaka. A total number of 44 apparently healthy Wister albino rats, weight between 150 to 200 grams; age ranging from 90 to 120 days was used. The rats were purchased from the animal house of Department of Pharmacy, Jahangir Nagar University, Shavar, Dhaka. The protocol of this study was approved by Institutional Ethics Committee (IEC) of DMC. The rats were kept in metallic case in the animal house of Institute of Nutrition and Food Science, University of Dhaka (DU). Prior conducting the study, rats were kept in a standard laboratory condition on a 12/12 hour light/dark cycle for 14 days acclimatization. All the rats received basal diet for 21 days. Total study period was 35 consecutive days and the work was done in the Institute of Nutrition and food science, DU. After selection, all the rats were acclimatized for 14 days. Then the rats were studied for 21 consecutive days. After acclimatization for 14 days, rats were divided into control groups (n=22) and experimental groups (n=22). Control groups again subdivided into BC (base line control group, n=11) and PC (paracetamol treated control group, n=11). Experimental groups were again subdivided into TCP-PCT (Terminalia chebula pretreated and paracetamol treated group, n=11) and PCP-TCT (paracetamol pretreated and Terminalia chebula treated group, n=11). After grouping, initial body weight of all the rats were measured on 1st day. All groups of rats received basal diet for 21 consecutive days. In addition to basal diet on 21st day, BC

received propylene glycol (2 ml/kg body weight, orally) and PC received single dose of paracetamol suspension (750 mg/kg body weight, orally). In experimental groups, TCP-PCT received Terminalia chebula extract (200 mg/kg body weight, orally) for 21 consecutive days and paracetamol suspension (750 mg/kg body weight, orally) on 21st day. Moreover, PCP-TCT received paracetamol suspension (750 mg/kg body weight, orally) on the 1st day and Terminalia chebula extract (200 mg/kg body weight orally) for 21 consecutive days. Powder form of paracetamol was purchased from Square pharmaceuticals and 1 gm of paracetamol was dissolved in 9 ml of propylene glycol and form paracetamol suspension. Again, 300 gm Terminalia chebula mixed with 800 ml distilled water for 3 days and form Terminalia chebula extract which stored in freeze at around 4^0C and was fed to the experimental rats. Before sacrifice, final body weights of all the rats were measured. On the 22nd day, all the rats were anaesthetized with the help of chloroform (30%) and then sacrificed. The blood samples (approximately 5 ml) were collected from the heart by direct puncturing by using sterile disposable syringes and taken in separate clean and dry test tubes with proper identification numbers. Then blood was centrifuged at a rate of 4000 rpm for 5 minutes. After that the supernatant serum was separated from the blood, collected in a labeled eppendorf and preserved in a refrigerator at -20ºc until analytical measurement of serum for total protein in Department of Pathology, DMC. Data was reported in Mean and SD (Standard deviation). Statistical analysis was done by One-way ANOVA test and Bonferroni test.

Results

The initial, final body weight of all rats were almost similar and showed no statistically significant difference between BC vs PC, PC vs TCP-PCT, TCP-PCT vs PCP-TCT (Table I).

The mean serum Alkaline phosphatase (ALP) level was significantly ($p<0.001$) higher in PC in comparison to that of BC. But this level was significantly ($p<0.001$) lower in TCP-PCT and PCP-TCT in comparison to that of PC. Again there was no significant difference in this level between TCP-PCT and PCP-TCT (Table II) and (Figure I).

Table I: Initial, Final and Percent (%) change of body weight in different groups of rats (n=44)

Parameters	BC (n=11)	PC (n=11)	TCP-PCT (n=11)	PCP-TCT (n=11)
Initial body wt(g) Day-1	158.18±6.03	156.45±6.35	161.18 ±14.37	157.91 ±9.85
Final body wt(g) Day-22	163.55±5.96	160.82±8.52	164.45 ±14.69	160.82 ±8.52
% change from final (F) weight to initial (I) weight [F-I/I×100]	3.39 ±1.26	2.80±1.8	2.02±1.25	1.84±2.21

Values are Means ± SD. Statistical analysis was done by one way ANOVA test. n = Number of rats. BC = Baseline control group PC = Paracetamol treated control group TCP-PCT =Terminalia chebula pretreated and paracetamol treated group PCP-TCT = Paracetamol pretreated and Terminalia chebula treated group.

Table II: Serum Alkaline Phosphatase (ALP) in different groups of rats (n=44)

Parameters	BC (n=11)	PC (n=11)	TCP-PCT (n=11)	PCP-TCT (n=11)
Total ALP(U/L)	97.09±20.93	163.09±26.93***	106.36±18.22¥¥¥	97.09±16.31¶¶¶

Values are Means ± SD. Statistical analysis was done by one way ANOVA test and then Bonferroni test. Serum Alkaline phosphatase (***p<0.001 BC vs PC) (¥¥¥p<0.001 TCP-PCT vs PC) (¶¶¶p<0.001 PCP-TCT vs PC). n = Number of rats BC= Baseline control group PC= Paracetamol treated control group TCP-PCT= Terminalia chebula pretreated and paracetamol treated group PCP-TCT= Paracetamol pretreated and Terminalia chebula treated group.

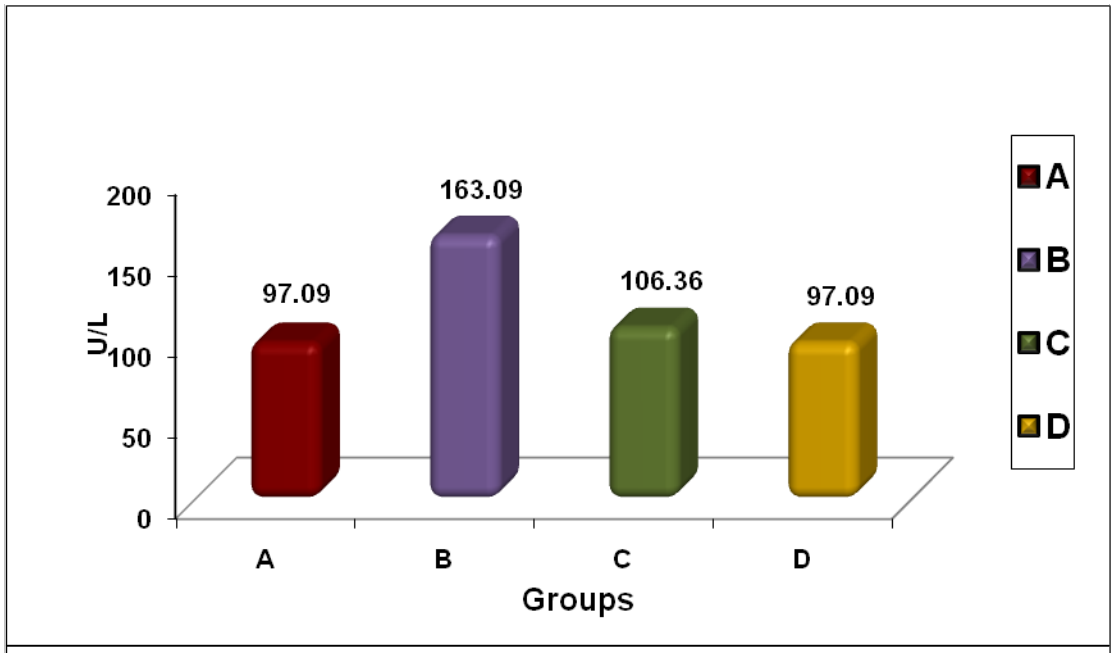

Fig 1. Mean Serum Alkaline Phosphatase (ALP) in different groups of rats (n=44)

n = Number of rats

Control group: Without Terminalia chebula
Group A: BC
Group B: PC
Experimental group: With Terminalia chebula
Group C: TCP-PCT
Group D: PCP-TCT

Discussion

In the present study, serum ALP level was significantly higher in PC in comparison to that of BC, TCP-PCT and PCP-TCT which is comparable to others.[14,15] But no significant change was observed by some researchers.[16] Different studies reported the toxic effect of high dose of paracetamol on hepatocytes and liver functions. It has been suggested that high doses of paracetamol disrupt the liver cell membrane causing increased release of ALP in the blood which is commonly used as a marker of liver function.[17] Some researchers suggested that metabolism of excess paracetamol in liver by conjugation with sulphate and glucuronide causes formation of toxic metabolites such as N-acetyl-p-benzoquineimine (NAPQI). This NAPQI causes oxidative stress by increasing the formation of reactive oxygen species which causes lipid peroxidation and depletion of antioxidant enzymes. This oxidative stress leads to destruction of structural and functional organization of cell membrane causing liver cell damage.[18] Moreover, high doses of paracetamol also causes activation of inflammatory mediator tumor necrosis factor- (TNF-) which secretes other cytokines and infiltrating neutrophils to release reactive oxygen intermediates that causes liver cell damage.[19] In this present study, liver damage was observed in rats treated with paracetamol as evidenced by their elevated levels of serum ALP. Some researchers suggested that Terminalia chebula contains some active compounds such as ellagic acid, chebulic acid and flavanoids which increase the activities of antioxidant enzymes which in turn obviously protect liver form oxidative damage[20]. Lower levels of serum ALP in TCP-PCT and PCP-TCT rats suggest that Terminalia chebula extract provides protection against paracetamol induced liver injury due to its free radical scavenging activity[21]. However, the exact mechanism involved in the hepatoprotective activity of Terminalia chebula extract against liver damage in rats cannot be explained out from this study as concentration of free radicals was not measured.

Conclusion

From this study, it can be concluded that Terminalia chebula (Haritaki) extract may have some hepatoprotective effect against paracetamol induced liver damage in rats. Therefore, it may be used to prevent liver damage with hepatotoxic drugs.

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