Hepatoprotective Effect of Terminalia Chebula (Haritaki) on Serum Bilirubin in Paracetamol Induced Liver Damage in Wister Albino Rats

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Liver plays a vital role in regulating various physiological processes. Any injury may lead to severe liver damage and impairment of liver function. 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 on paracetamol induced change of serum bilirubin in Wister albino rats. This experimental study was carried out in the Department of Physiology, Dhaka Medical College, Dhaka from January 2013 to December 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). All groups of rats received basal diet for 21 consecutive days. In addition to basal diet, rats of BC received propylene glycol (2ml/kg body weight, orally) and PC received single dose of paracetamol suspension (750mg/kg body weight, orally) on 21st day. Rats of TCP-PCT received Terminalia chebula extract (200 mg/kg body weight, orally) for 21 consecutive days and paracetamol suspension (750mg/kg body weight, orally) on 21st day. Moreover, rats of PCP-TCT received paracetamol suspension (750mg/kg body weight, orally) on the 1st day and Terminalia chebula extract (200 mg/kg body weight orally) for 21 consecutive days. 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 bilirubin 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 bilirubin level was significantly ($p<0.001$) higher in paracetamol treated control group in comparison to those of baseline control group. Serum bilirubin level of all experimental groups were significantly ($P<0.001$) lower than PC group. **Conclusion:** From the results of this study, it may be concluded that Terminalia chebula may have some hepatoprotective effects in paracetamol induced liver damage in rats.

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Key words: Terminalia chebula, hepatoprotective, paracetamol, bilirubin

Introduction

Liver is an essential for life because it performs numerous functions such as metabolic functions, synthesis of proteins, formation and secretion of bile, regulation of blood glucose level, detoxification of drugs and endogenous compounds and storage of vitamins and minerals.[1] Continuous exposure and intoxication of liver to different types of exogenous compounds may lead to liver

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dysfunction.[2] Liver diseases are major health problem worldwide, with high endemicity in developing countries. About 20,000 deaths occur every year due to liver diseases.[3] The modern or synthetic drugs used in the treatment of liver diseases have been reported to cause serious adverse side effects. It has been reported that alternative natural sources of medicinal plants have less or no side effect.[4] 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 availability of paracetamol have led to misuse of the drug and may cause a number of serious clinical problems.[5] Paracetamol is hepatotoxic when used in excessive doses or when used in therapeutic doses for a prolonged period.[6,7] Conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes may have serious side effects. But there are a number of medicinal preparations with less side effects are present in Ayurveda and recommended for the treatment of liver disorders. In medicinal practices, reliable liver protective drugs are not available but herbs may play an important role in management of liver disorders.[8]

Terminalia chebula have been used in folk medicine throughout the ancient times in Bangladesh. It is locally known as Haritaki and a member of Combretaceae family. Terminalia chebula is also called as king of medicine because it has been widely used in ayurveda, unani, siddha and homeopathy.[9] Terminalia chebula contains tannin, chebulic acid, glycosides, sugar, triterpenoids, steroids and small quantity of phosphoric acid. Many studies have been reported for biological properties of Terminalia chebula and it has effects on different diseases.[10] Till today, no side effects have been found in Terminalia chebula. Different researchers from different countries have been studied the hepatoprotective effects of Terminalia chebula.[11] Recently, some investigators observed that Terminalia chebula significantly decreased the paracetamol induced elevation of serum bilirubin in rats.[12,13] Now a day there is increasing need for substances to protect the liver from damage. Modern medicines have more side effects to cure liver diseases but remedies by medicinal plants have lesser side effects for the treatment of liver diseases. Therefore, to investigate the hepatoprotective properties of natural substances the present study has been designed to examine the hepatoprotective effect of Terminalia chebula on paracetamol induced liver damage in Wister albino rats.

Methods

This experimental study was conducted between 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 in to 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 21th day, BC received propylene glycol (2ml/kg body weight, orally) and PC received single dose of paracetamol suspension (750mg/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 21th 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 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 22th 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 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 bilirubin 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 and % changes of 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).

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 bilirubin in different groups of rats (n=44)

Parameters	BC (n=11)	PC (n=11)	TCP-PCT (n=11)	PCP-TCT (n=11)
Bilirubin(mg/dl)	0.36±0.11	1.21±0.42***	0.65±0.34$^{¥¥¥}$	0.61±0.21¶¶¶

Values are Means ± SD. Statistical analysis was done by one way ANOVA test and then Bonferroni test. Serum bilirubin (***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.

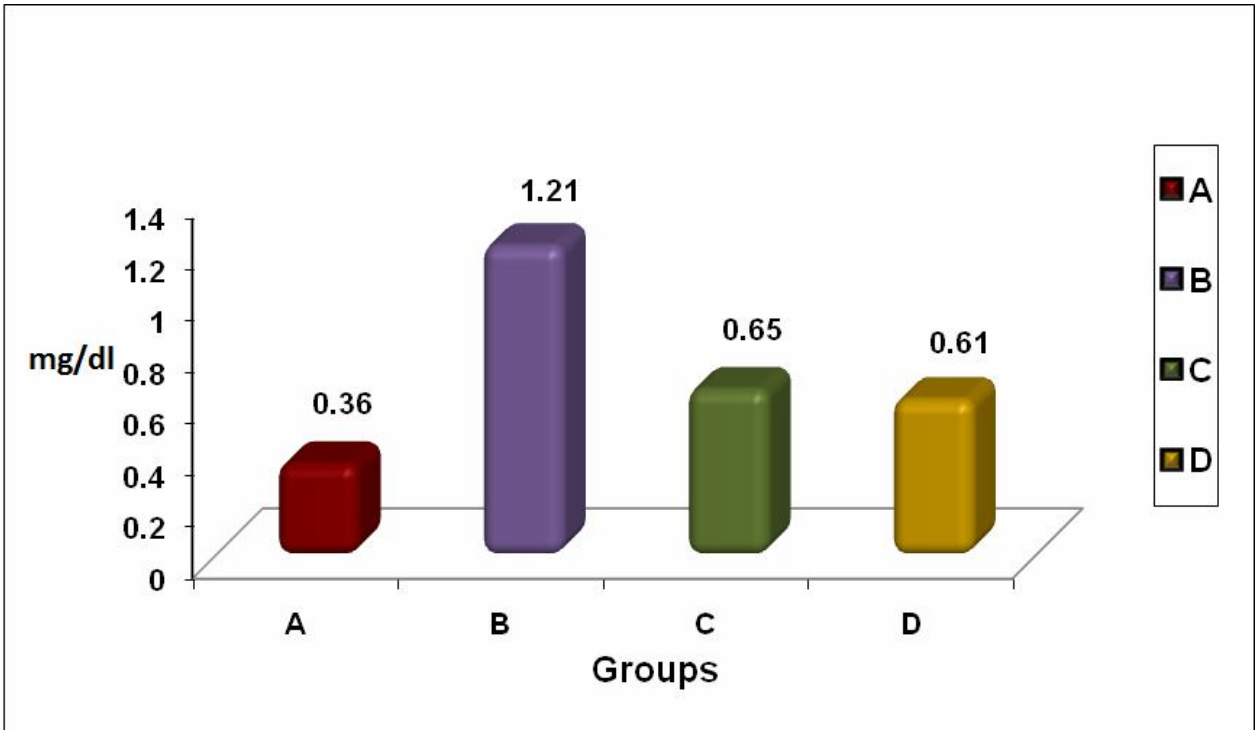

Figure 1. Mean serum bilirubin in different groups of rats (n=44)

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

The mean serum bilirubin 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 1).

Discussion

In the present study, serum bilirubin level was significantly higher in PC in comparison to that of BC, TCP-PCT and PCP-TCT which is comparable to others.[14,15,16] But no significant change was observed by some researchers.[17] Different studies reported the toxic effect of high dose of paracetamol on hepatocytes and liver function. It has been suggested that high doses of paracetamol disrupts the liver cell membrane causing increased release of bilirubin in the blood which are commonly used marker of liver function.[18] It has also been 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 imposes oxidative stress by increasing the formation of reactive oxygen species which causes lipid peroxidation and depletion of antioxidant enzymes. This increased oxidative stress leads to destruction of structural and functional organization of cell membrane causing liver cell damage.[19] Moreover, high doses of paracetamol also oxidizes intracellular glutathione (GSH) which causes GSH pool depletion in liver cell leading to liver cell damage.[18] In this study elevated level of serum bilirubin is suggestive of liver cell damage. Studies on medicinal plants demonstrated that Terminalia chebula contains some active compounds such as vitamin C, ellagic acid, gallic acid, chebulic acid, bellericanin, β-sitosterol and flavanoids which increase the activities of antioxidant enzymes which in turn obviously protect liver for oxidative damage.[20] Lower levels of serum bilirubin 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.[10] 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) may have some hepatoprotective role against paracetamol induced liver damage. Therefore, it may be used to prevent liver damage with hepatotoxic drugs.

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