# Hepatoprotective activity of Ethanolic extract of leaf and seed of *Tamarindus Indica* against Paracetamol induced Hepatotoxicity in Rats.

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**ABSTRACT:**

**Background:** In Bangladesh about 35million people are suffering from liver disease. Herbs play a vital role in the management of various liver disease in absence of reliable hepatoprotective drugs in Allopathic medical practice. **Objective:** to observe the hepatoprotective effect of ethanolic extract of *Tamarindus Indica* leaves & seeds. **Methods:** A total of 24 male Long Evan's rats (150-180gm) were used for this study. They were divided into 4 groups designated as control received normal diet *ad libitum only*, Only paracetamol group received paracetamol (1500 mg/kg) for 12 days and rest two groups ethanolic extract of leaf and seed received paracetamol (1500mg/kg) along with ethanolic extract of leaf (1250 mg/kg) and seed (1250 mg/kg) of *Tamarindus indica* for 12 days respectively. After 24 hours of last treatment, all rats were sacrificed for the estimation of liver function parameters (ALT, AST, ALP and bilirubin) and histopathological study of their liver tissue.Ethical clearance for the use of animals was obtained from the committee constituted for the purpose. **Results:** Only Paracetamol group showed a significant increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and alkaline phosphatase (ALP) along with gross hepatocellular damage. On the other hand ethanolic leaves(1250mg/kg) and seeds (1250mg/kg) extract of *Tamarindus Indica* treated groups showed a significant decrease of the above parameters and histopathological examination of liver tissue revealed an almost return to normal architecture. The values were expressed as mean ± SD. The statistical analysis was carried out by unpaired student's "t" test and $P<0.05$ was considered as significant. **Conclusion:** *Tamarindus Indica* a very commonly used food can be applied for treatment of drugs or chemical induced hepatotoxicity.

## INTRODUCTION

Liver is the major site of intense metabolic activities. Because of its strategic anatomical location, it is exposed to many kinds of xenobiotics and therapeutic agents. Certain medicinal agents like Paracetamol, Aspirin, Halothane, Diclofenac etc. when taken in overdoses or within therapeutic ranges may be the cause of liver injury.[1] Despite of excellent regeneration capacity of this organ, a slight injury or toxicity may lead to fatal complications.[2] Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences. In Bangladesh about 35million people are suffering from liver disease[3] Unfotunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects.[4] In the absence of reliable hepatoprotective drugs in Allopathic medical practice, herbs play a vital role in the management of various liver disease.[5] Several studies have been carried out to see hepatoprotective effect of plants such as *Feronia elephantum*[6], *Aerva Lanata*[4], *Costus Speciosus*[7], *Cleome Viscosa*.[3]

*Tamarindus Indica*, commonly known as tentul is a tree type plant belonging to the family Fabaceae. It is indigenous to tropical Africa and also cultivated in Bangladesh, India, Pakistan, Sudan, Java, Philippines, Indonesia and Spain. The fruit of tamarind is a digestive aid, laxative , expectorant, blood tonic. Other parts of plant show antioxidant[20], antihepatotoxic[9] ,antidiabetic[10], antiinflammatory[11] activities .

Hepatoprotective effect of *Tamarindus Indica* has also been studied using aqueous extract of leaf[12], fruit[13], seed.[12] Results of these studies showed that all of these are hepatoprotective. Studies suggest that hepatoprotective effect of *Tamarindus indica* is due to presence of flavonoids, ascorbic acid, β-carotene. This study was undertaken to see hepatoprotective effect of ethanolic extract of leaf & seed of *Tamarindus Indica* from paracetamol induced hepatotoxicity in rats.

## METHODS

### Plant Materials:

Leaves and seeds of *Tamarindus Indica* procured from Sirajganj and identified by plant taxonomy unit of Bangladesh National Herbarium with voucher specimen no. DACB-35524, which was deposited to herbarium.

### Preparation of Plant extract:

Leaves and seeds were shade dried and powdered by electric blender and grinder machine. The powders were soaked separately in 95% ethanol. The extract so obtained was concentrated in vaccum rotatory

evaporator at 40-50°c until a paste was formed. Extract paste was freeze dried.

**Drugs and Chemicals:**
Paracetamol powder was obtained from Kumudini Pharmaceuticals Ltd., Bangladesh. Propylene Glycol was used as solvent for paracetamol powder.

**Animals:**
A total of 24 male Long Evan's rats (150-180gm) were used for this study. The animals were kept in well ventilated room in the animal house of Dhaka Medical College. A 12hr light / 12 hr dark cycle was maintained. They were given standard food pellets and allowed drinking water *ad libitum*. Ethical clearance for the use of animals was obtained from the committee constituted for the purpose.

**Procedure:**
A total of twenty four rats were taken and divided into four groups. Each group having six rats (n=6). Control group received normal diet *ad libitum*. Only paracetamol group received paracetamol (1500 mg/kg) for 12 days and two groups received paracetamol (1500mg/kg) along with ethanolic extract of leaf (1250 mg/kg) and seed (1250 mg/kg) of *Tamarindus indica* respectively for 12 days.

After 24 hours of last treatment, all rats were anaesthetized with light chloroform and blood were collected by Cardiac puncture and serum was separated for estimations of ALT, AST, ALP and bilirubin.

**Histopathological Study:**
Liver tissue was taken and fixed in 10% formalin and sections of liver tissue were embedded in paraffin and made blocks. Serial sections of blocks 3μ-5μ thickness were made and stained with Haematoxylin and Eosin and examined under microscope.

**Statistical Analysis:**
The values were expressed as mean ± SD. The statistical analysis was carried out by unpaired student's "t" test and $P<0.05$ was considered as significant.

**Result**
In rats treated with only paracetamol serum bilirubin, ALT, AST, ALP increased significantly as compared to control group. Significant decrease in serum bilirubin, ALT, AST, ALP was observed following administration of ethanolic extract of leaf & seed of *Tamarindus Indica* along with paracetamol (Table1). Histopathological examination of liver tissue in Paracetamol administered rats showed massive liver tissue necrosis with loss of cellular architecture and infiltration of neutrophil, macrophage and lymphocyte (Fig: 2). An almost return to normal architectutre of hepatic tissue was observed in rats that received ethanolic extract of leaf & seed of *Tamarindus Indica* (Fig : 3 & 4) along with paracetamol.

Table 1: Serum bilirubin, ALT, AST and ALP level in rats that received Paracetamol along with ethanolic extract of leaf and seed of Tamarindus Indica. (12 days)

| Groups | Serum bilirubin (mg/dl) | Serum ALT (U/L) | Serum AST ( U/L) | Serum ALP (U/L) |
|---|---|---|---|---|
| Control | 0.51±0.16 | 43.33±3.20 | 38.367±3.56 | 98.00±14.03 |
| Only Paracetamol (1500mg/kg ) | 0.93±0.20** | 162.66±5.60*** | 144.66±7.78*** | 378.00±22.44*** |
| Paracetamol (1500mg/kg)+ leafextract*T.indica* (1250mg/kg) | 0.53±0.16* | 63.16±5.04*** | 53.00±5.09*** | 202.00±25.64*** |
| Paracetamol (1500mg/kg)+ seedextract*T.indica* (1250mg/kg) | 0.67±0.16* | 63.50±3.62*** | 57.83±6.18*** | 192.00±21.47*** |

n = 6. All the drugs were administered orally through ryles tube. Data expressed as mean ± SD. ***P<0.001 taken as significant.

**Discussion:**

Significant increase in serum bilirubin, ALT, AST and ALP following administration of paracetamol is an indicator of hepatotoxicity. Assessment of liver damage can be assessed by estimation of serum ALT, AST and ALP.[12] Necrosis results in the release of these enzymes into circulation, therefore, it can be measured in serum. High levels of AST indicate liver damage, ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manners, therefore, ALT is more specific to liver and is thus a better parameter for detecting liver damage.[14]

The results in present study showed significant damage to liver tissue following administration of paracetamol, confirmed by histopathological examination of liver tissue that showed massive necrosis and infiltration of macrophage and lymphocyte.

Paracetamol causes acute liver damage[15] due to excessive formation of highly reactive metabolite N-acetyl Parabenzoquinone-imine (NAPQI)[16], which conjugates with hepatic glutathone. Hepatic glutathone is depleted because of ingestion of large dose of paracetamol. NAPQI arylates essential nucleophilic macro molecules within hepatocytes, forming stable acetaminophen-protein adducts which are responsible for Acetaminophen induced hepatotoxicity.[17] Elevated enzymes level showed loss of functional integrity of hepatocytes.[18] Groups treated with ethanolic leaves and seeds extract of *Tamarindus Indica* showed significant change in liver architecture and tissue necrosis as revealed in histopathology which gradually became normal. The changes in biochemical values were consistent with the histopathological findings. Similar other studies have been carried out using aqueous extract of leaf[12], seed[12], fruit[13] extract of *Tamarindus Indica*. Results of these studies using water extract was almost similar to ethanolic extract of *Tamarindus Indica*. Hepatoprotective effect of *Tamarindus Indica* has been attributed due to presence of flavonoids, polyphenol, β-carotene, ascorbic acid.[19] A number of scientific reports indicated that flavonoids, β-carotene, ascorbic acid have protective effect on liver due to their antioxidant properties.[20]

Therefore *Tamarindus Indica* a very commonly used food can be applied for treatment of drugs or chemical induced hepatotoxicity. *Tamarindus Indica* is easily available, cheap and its fruit is consumed as popular food. Before clinical application further studies on its hepatoprotective effect might be carried out.

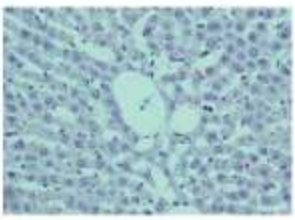

Fig-1 : Photomicrographs (Magnification at 40X objectives) showing the normal hepatic architecture in control group.

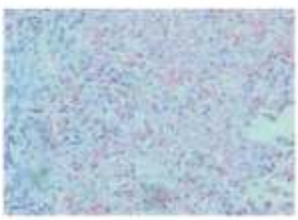

Fig-2 : Photomicrographs (Magnification at 40X objectives) showing Paracetamol induced hepatic necrosis and small number of inflammatory cells.

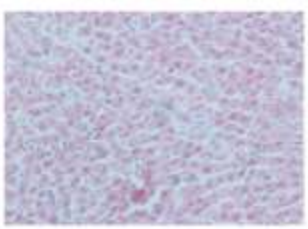

Fig-3: Photomicrographs (Magnification at 40X objectives) showing normal hepatic architecture following administration of leaf extract of *Tamarindus indica* along with Paracetamol.

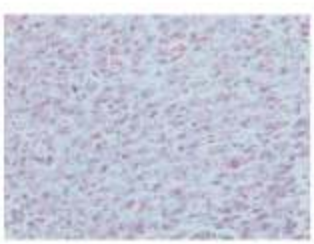

Fig-4: Photomicrographs (Magnification at 40X objectives) showing normal hepatic architecture following administration of seed extract of *Tamarindus indica* along with Paracetamol.

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