

PROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF LEAF AND SEED OF *TAMARINDUS INDICA* ON PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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Abstract:

Protective effect of ethanolic extract of *Tamarindus Indica* leaves & seeds were studied on paracetamol induced hepatotoxicity in Long Evan's Rats. Different groups of animal were administered paracetamol (1500mg/kg, p.o.) for 7 days. Ethanolic extract of leaves and seeds at the dose of 1250mg/kg were administered to paracetamol pretreated rats. On treatment with paracetamol a significant increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and alkaline phosphatase was observed. On administration of ethanolic extract of leaves and seeds a significant decrease in the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and alkaline phosphatase (ALP) was observed and histopathological examination of liver tissue revealed an almost return to normal architecture.

J Dhaka Med Coll. 2012; 21(1) : 12-15.

Introduction:

Liver is the main organ of biotransformation in the body and vulnerable to ingested xenobiotics. Acute liver disease due to various reasons is a common cause of morbidity and mortality. Severe acute liver disease is encountered in clinical practice leading to fulminant or acute liver failure¹. Most common cause of fulminant hepatic failure include drug or toxin induced hepatic injury or viral hepatitis². There are some 900 drugs that can cause liver damage. Some of these medicinal agents when taken in therapeutic dose maybe the cause of liver injury, whereas most of them in over dose are definitely injurious to liver. Incidence of fatality due to drug induced liver damage is very high. Some of the commonly used analgesics like Aspirin, Halothane, Methyldopa, Acetaminophen etc. cause hepatotoxicity.

In Bangladesh about 35million people are suffering from liver disease³ and 80% of death is due to fulminant hepatic failure⁴.

Though liver disease is a worldwide problem, available remedies are scarce. Several studies have been carried out to see hepatoprotective effect of plants such as *Semecarpus anacardium*⁵, *Aerva Lanata*⁶, *Costus Speciosus*¹⁰, *Cleome Viscosa*⁷.

Hepatoprotective effect of *Tamarindus Indica* (Family-Caesalpinaceae) commonly known as tentul has also been studied using aqueous extract of leaf⁸, fruit⁹, seed⁸. 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, b-carotene. In the present study ethanolic extract of leaf & seed of *Tamarindus Indica* were used on paracetamol induced liver damage in rats.

Materials & methods:

Plant Materials:

Leaves and seeds of *Tamarindus Indica* procured from Sirajganj and identified by plant taxonomy

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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 vacuum 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.

Methodology:

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 7 days and two groups received paracetamol (1500 mg/kg) for first 7 days followed by ethanolic extract of leaf

(1250 mg/kg) and seed (1250 mg/kg) of *Tamarindus indica* for next 5 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 pretreated with paracetamol serum bilirubin, ALT, AST, ALP increased significantly as compared to control group (Fig: 1 & 2). Significant decrease in serum bilirubin, ALT, AST, ALP was observed following administration of ethanolic extract of leaf & seed of *Tamarindus Indica* (Fig: 3 & 4). 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 architecture of hepatic tissue was observed in rats that received ethanolic extract of leaf & seed of *Tamarindus Indica* (Fig : 3 & 4).

Table-I

Serum bilirubin, ALT, AST and ALP level in Paracetamol pretreated (7 days) rats that received ethanolic extract of leaf and seed of Tamarindus Indica.

Groups	Serum bilirubin (mg/dl)	Serum ALT (U/L)	Serum AST (U/L)	Serum ALP (U/L)
Control	0.450±0.13	41.33±4.45	36.33±3.44	90.00±12.59
Only Paracetamol (1500mg/kg)	1.06±0.48**	193.00±12.87***	166.00±19.67***	428.00±33.66***
Leaf extract <i>T. indica</i> (1250mg/kg)	0.65±0.17*	61.33±2.50***	50.33±1.63***	174.00±22.45***
Seed extract <i>T.indica</i> (1250mg/kg)	0.57±0.20*	60.17±4.54***	53.00±5.59***	178.00±27.80***

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

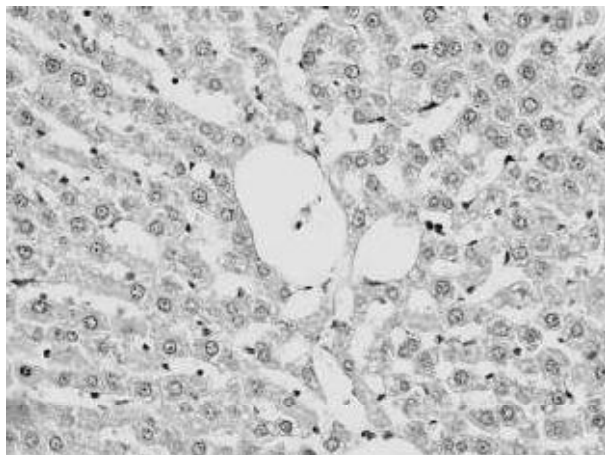


Fig-1 : Photomicrographs (Magnification at 40X objectives) showing the normal hepatic architecture in control group.

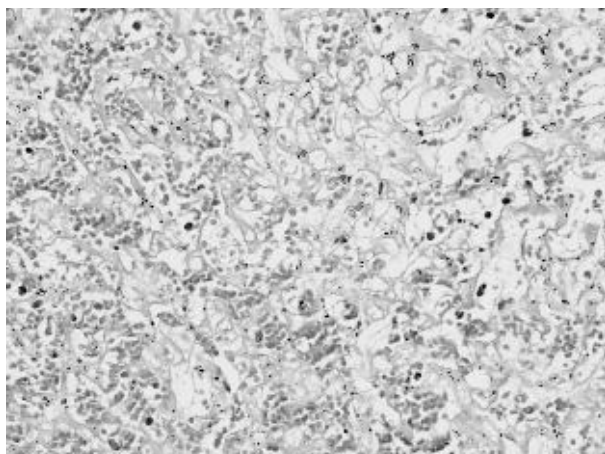


Fig-2 : Photomicrographs (Magnification at 40X objectives) showing Paracetamol induced hepatic necrosis and small number of inflammatory cells.

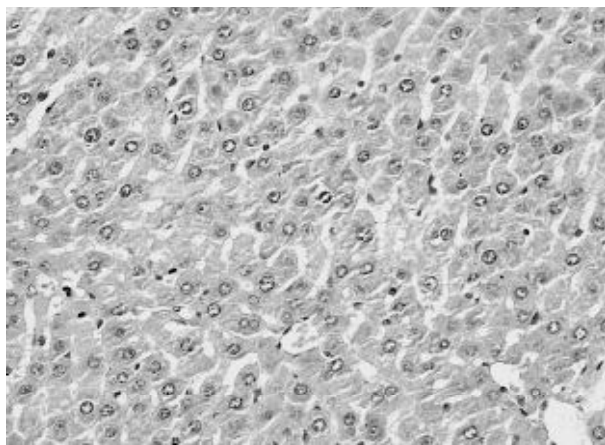


Fig-3: Photomicrographs (Magnification at 40X objectives) showing normal hepatic architecture following administration of leaf extract of Tamarindus indica in Paracetamol induced hepatotoxicity.

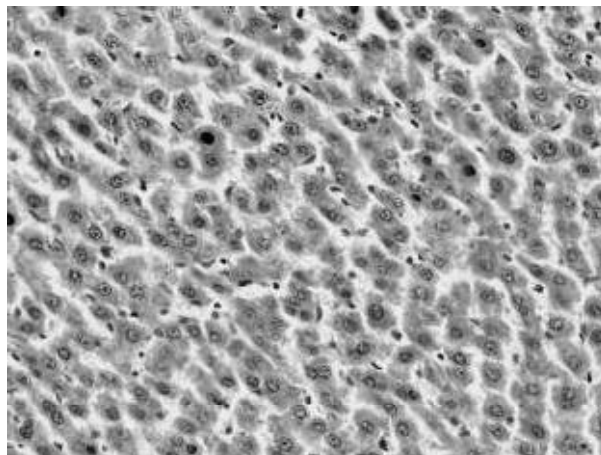


Fig-4: Photomicrographs (Magnification at 40X objectives) showing normal hepatic architecture following administration of seed extract of Tamarindus indica in Paracetamol induced hepatotoxicity.

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 ALP⁸. 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 damage¹¹.

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 damage¹⁴ due to depletion of glutathione and formation of highly reactive metabolite N-acetyl Parabenzoquinone-imine (NAPQI)¹³. NAPQI arylates essential nucleophilic macro molecules within hepatocytes, forming stable acetaminophen-protein adducts which are responsible for Acetaminophen induced hepatotoxicity¹². Elevated enzymes level showed loss of functional integrity of

hepatocytes¹⁵. Administration of ethanolic extract of leaf & seed of *Tamarindus Indica* decrease liver enzymes level and return of liver tissue to almost normal architecture on histopathological examination is suggestive of its hepatoprotective effect. Similar other studies have been carried out using aqueous extract of leaf⁸, seed⁸, fruit⁹ 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, b-carotene, ascorbic acid¹⁷. A number of scientific reports indicated that flavonoids, b-carotene, ascorbic acid have protective effect on liver due to their antioxidant properties¹⁶.

Therefore *Tamarindus Indica* a very commonly used food can be applied for treatment of drugs or chemical induced hepatotoxicity. Use of natural product cause less adverse effect compared to synthetic analogues. *Tamarindus Indica* is easily available, cheap and its fruit is consumed as popular food. Before clinical application further future studies on its beneficial hepatoprotective effect might be carried out.

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