

Hepato-Protective Activity of *Phyllanthus emblica* Against Paracetamol Induced Hepatic Damage in Wister Albino Rats

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Abstract: The efficacy of the medicinal plant *Phyllanthus emblica* to prevent paracetamol induced hepatotoxicity in rats was examined by the histopathological study of liver cells. The total blood cell count in each group of animal was also calculated. Treatment with aqueous extract of fruits of *Phyllanthus emblica* showed the appearance of normal hepatocytes, offset of necrosis and consequent appearance of leucocytes thus suggesting the hepatoprotective effect of this medicinal plant.

Key words: Paracetamol • *Phyllanthus emblica* • Histopathology

INTRODUCTION

Medicinal plants play an important role in the management of diseases. In India almost 45, 000 plant species are growing naturally and being cultivated. One among them is Indian gooseberry (*Phyllanthus emblica*), which is used as hepatoprotective agent.

Paracetamol or acetaminophen is the active metabolite of phenacetin, a so called coal tar analgesic is widely used as an analgesic and antipyretic drug [1]. It is administered in tablet, liquid suspension and suppository, intravenous or intramuscular form. The common adult dose is 500-1000 mg. It is considered safe for human use at recommended doses; however acute overdose can lead to potentially lethal liver and in severe cases leads to death [2-5]. In general administration of paracetamol damage hepatocytes as they metabolise the paracetamol. Rarely acute renal failure also may occur. In spite of this the intake of paracetamol became inevitable because of today's life. In human adult single dose above 10 grams or 150 mg/kg is found to be toxic [6]. In children acute doses above 200 mg/kg could cause toxicity. Paracetamol has also been shown to promote hepatocyte apoptosis [7].

Weighing about 2-3 pounds, the liver is a vital organ which performs numerous functions like metabolism of carbohydrate, protein and fats and detoxifying substances that would otherwise be harmful to the body.

Liver diseases remain to be serious health problems and management of liver disease is still a challenge to the modern medicine. The liver occupies the pivotal position in body plays an essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism. The role played by this organ in the removal of toxic substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign (xenobiotic) compounds culminating in liver dysfunction [8]. Despite the tremendous strides in modern medicine there is still a need for a drug that stimulates liver function or offers protection to the liver from damage or helps regeneration of hepatic cells [9].

Medicinal plants such as *Andrographis paniculata*, *Eclipta alba*, *Phyllanthus amarus*, *Phyllanthus debilis* and *Boerhaavia diffusa* are well known for their hepatoprotective effects [10-13]. Fruits of *Phyllanthus emblica* is commonly used in the treatment of anorexia, diarrhoea [14], jaundice, cough [14, 15], ulcer [16, 17].

The fruit of *Phyllanthus emblica* has been used for thousands of years by Ayurvedic Physicians who refer it as the "Sustainer" and "the fruit where the goddess of prosperity resides". Although *Phyllanthus emblica* is reputed to have the highest content of Vit.C, recent scientific interest has centered on its unique tannins and flavanoids which contain very powerful antioxidant properties and it is a potent immune stimulator since it activates natural killer (NK) cell activity [18]. Many other

interesting and useful activities of *Phyllanthus emblica* have recently been confirmed which includes anti-tumor, anti-inflammatory, anti-bacterial, liver and cardiovascular protective properties [19].

Hence a study was undertaken to investigate the hepato protective effects of *Phyllanthus emblica* fruit extract on paracetamol induced rat hepatic injury.

MATERIALS AND METHODS

Collection of Plant Materials: Fruits of *Phyllanthus emblica* were obtained from Marthandam market, kanyakumari district, Tamil Nadu, India during Dec.2007.

Preparation of Extract: Fruits of *Phyllanthus emblica* were shade dried for a week, powdered mechanically, sieved (sieve no: 10/44) and stored in air tight containers. About 250 g of the powdered material is boiled in 500 ml distilled water for 30 minutes, kept for 3 days with intermittent shaking, filtered and concentrated to get the completely dried aqueous extract.

Animals: Adult Wister Albino rats weighing 170-175 g were procured from Prasanth aquarium, Marthandam. The animals were housed in polypropylene cages and maintained at $28 \pm 2^\circ\text{C}$, $70 \pm 5\%$ RH and at 12 hr light/dark photoperiod. They were fed with ordinary natural feed (rice powder and cooked rice) and water *ad libitum* during the experiment.

Evaluation of Hepatoprotective Activity: The animals were divided into four groups of four rats each. The animals in group-1 served as control. All the animals of group-2 to group-4 received 2g/kg, bw, po (per oral) paracetamol for 14 days. Aqueous extracts of 100 and 200 mg/kg/day, po of *Phyllanthus emblica* were administered to the animals of group-3 and group-4 respectively for the same 14 days. The animals of all the groups were sacrificed by diethyl ether anaesthesia on the 14th day. The blood sample of each animal was collected separately by carotid bleeding into sterilized dry tubes and was tested for erythrocyte (RBC) and leucocyte (WBC) counts using Neubauer counting chamber.

Histopathology: The liver samples were excised from the experimental animals of each group and washed with normal saline. Initially the materials were fixed in 10% buffered neutral formalin for 48 hrs and then with bovine

solution for 6 hrs. They were then processed for paraffin embedding. The sections were taken at 5µm thickness using microtome, processed in alcohol-xylene series and stained with alum-haematoxylin and eosin [20]. The sections were examined microscopically for the evaluation of histopathological changes.

RESULTS

Acute Toxicity Study: In the acute toxicity study the rats administered with paracetamol (2g) showed more lethality and toxic effects. The rats administered with both paracetamol and *Phyllanthus emblica* fruit extract showed better results. Hepatoprotective agent *Phyllanthus emblica* at a concentration of 200mg/kg along with paracetamol showed positive significant results than that at a concentration of 100mg/kg along with paracetamol.

Mortality: In control group no mortality was observed. 50% mortality was observed in both group-2 and 3. 25% mortality was observed in group-4 (Table 1). Percentage mortality (%) is calculated by,

$$\text{Mortality (\%)} = \frac{\text{No. of individuals died}}{\text{Total No. of individuals in one group}} \times 100$$

Blood Cell Count: The total blood cells in each group of animals were counted. In control group of animals normal counts of WBCs (4850 cells/cm.mm) and RBCs (4.24 million/cm.mm) were observed. There was a vast deviation in the blood cell count (WBCs 2750 cells/cm.mm and RBCs 4.14 million/cm.mm) of animals administered with paracetamol (2g) alone. Animals treated with paracetamol (2g) along with *Phyllanthus emblica* (100mg and 200mg) showed normal blood counts (Table 2).

Table 1: Mortality

Group	Mortality (%)
Control (Group - 1)	0
(Group - 2)	50%
(Group - 3)	50%
(Group - 4)	25%

Table 2: Blood Cell Count

Groups	Total leucocytes count	Total erythrocytes count
Group - 1	4850 cells / cm.mm	4.24 million / cm.mm
Group - 2	2750 cells/cm.mm	4.14million/cm.mm
Group - 3	4450 cells/cm.mm	5.51 million /cm.mm
Group - 4	4950 cells/cm.mm	4.39 million/cu.mm

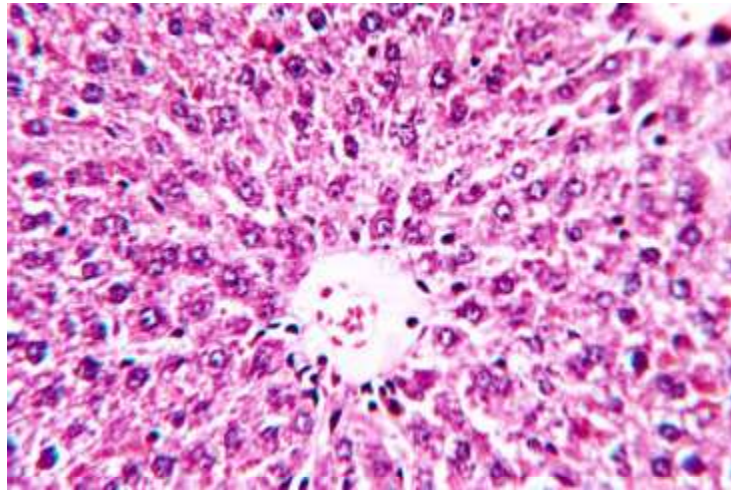


Fig. 1: Histology of Liver (Control group)

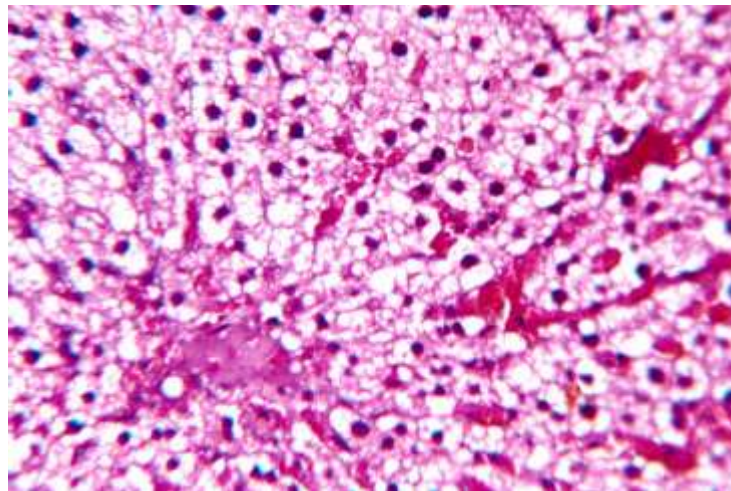


Fig. 2: Vacuolated Liver with heavy bleeding (group 2)

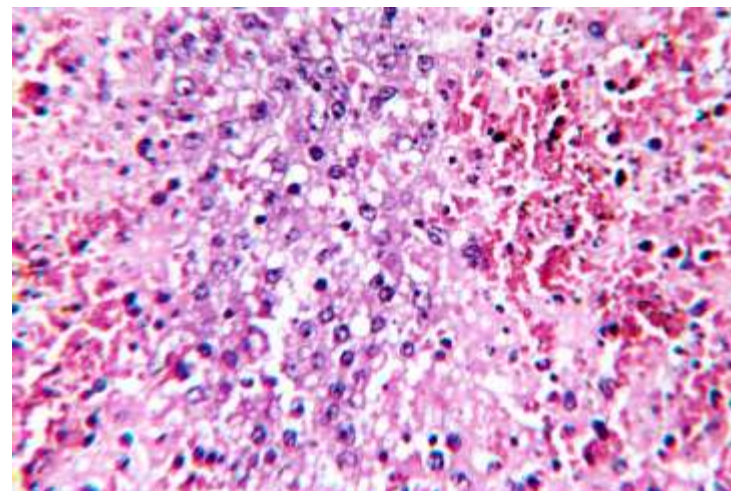


Fig. 3: A portion of Liver with normal and affected areas (group 3)

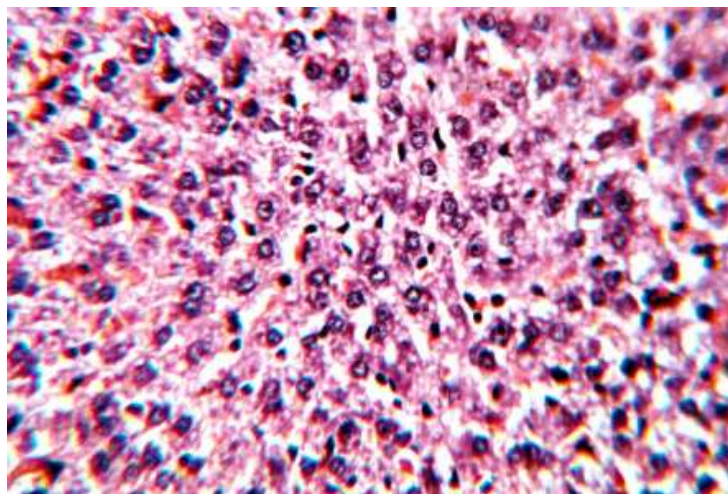


Fig. 4: Area of Liver with normal hepatocytes (group 4)

Histological Changes: The histoarchitecture of control group animals showed normal cells (Fig 1). The histoarchitecture of paracetamol treated rat liver sections showed cloudy swelling and fat cells, degeneration of hepatocytes and heavy haemorrhage. Necrosis of cells was also observed with complete degeneration of liver cells, broken cell pieces, irregular appearance due to oozing of cell materials and cell death (Fig 2). In the group of animals treated with paracetamol and *Phyllanthus emblica* (100mg/kg), normal and affected areas of liver in patches adjacent to each other, was observed (Fig 3). The degenerative changes, necrosis, haemorrhage were less observed and consequent appearance of leucocytes which is essential for self immunity is observed in the group of rats treated with *Phyllanthus emblica* (200mg/kg) thus confirming the hepatoprotective effect (Fig 4). These results provide evidence to *Phyllanthus emblica* as a hepatoprotective agent.

DISCUSSION

A lot of medicinal plants, traditionally used for thousands of years, by the Indian traditional health care system (ayurvedic) named 'Rasayana' for their antioxidative properties. *Phyllanthus emblica* was a very good antioxidant and hepatoprotective agent [21]. *Phyllanthus emblica* (100-200mg/kg) increased cell viability of rat hepatocytes being treated with paracetamol (2g). Pretreatment of rats with *Phyllanthus emblica* at oral doses of 100-200mg/kg, 4 hrs before paracetamol administration lowered the hepatotoxicity. Paracetamol is also toxic to cats and proved fatal [22]. Paracetamol is lethal to snakes also and has been used in attempts to

control the brown tree snake [23]. Paracetamol consumed with coffee can be dangerous to health [24]. The tannins and flavanoids present in *Phyllanthus emblica* fruit extract contain very powerful antioxidant and hepatoprotective properties [16, 25]. Present experiment also indicates that animals administered with paracetamol (2g) alone showed higher hepatotoxicity. The rats administered with paracetamol and *Phyllanthus emblica* showed protective effects in the liver.

These finding shows that *Phyllanthus emblica* fruit extract have the ability to rectify hepatic damage or toxicity. Hence it is advised that if one happens to take paracetamol in overdose they can consume *Phyllanthus emblica* fruit extract as a hepatoprotective agent. Thus always have in mind that "Prevention is better than cure".

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