Indian J Physiol Pharmacol 2000; 44 (1): 64–68

EFFECT OF *AZADIRACHTA INDICA* (NEEM) LEAF AQUEOUS EXTRACT ON PARACETAMOL–INDUCED LIVER DAMAGE IN RATS

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(Received on April 10, 1999)

Abstract : The effect of aqueous leaf extract of *Azadirachta indica* (A. indica) was evaluated in paracetamol induced hepatotoxicity in rats. Liver necrosis was produced by administering single dose of paracetamol (2 g/kg, p.o.). The liver damage was evidenced by elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (γ-GT) and by histopathological observations of liver sections. Aqueous *A. indica* leaf extract (500 mg/kg, p.o.) significantly ($P<0.01$) reduced these elevated levels of AST, ALT and γ-GT. Paracetamol induced liver necrosis was also found to be reduced as observed macroscopically and histologically.

Key words : paracetamol hepatotoxicity hepatoprotective *Azadirachta india*

INTRODUCTION

Azadirachta indica (A. indica), commonly known as Neem is reported to possess anti-inflammatory (1), hypoglycaemic (2), hypolipidaemic (2) and immunostimulant (3) properties. Chattopadhyay et al (4) reported hepatoprotective activity of alcoholic extract of leaves of A. indica in rats. Sen et al (3) have reported a decrease in SGOT and SGPT levels in A. indica treated animals who were subjected to restraint stress. However, very few studies (3, 4) are available to evaluate the hepatoprotective effect of A. indica. The present study was undertaken to evaluate the hepatoprotective effect of aqueous extract of A. indica leaves by employing paracetamol as hepatotoxic agent.

Paracetamol induced hepatotoxicity is thought to be caused by N-acetyl-p-benzoquinoneimine (NAPQI), a cytochrome P450 mediated intermediate metabolite (5). NAPQI can react with sulphydryl groups such as glutathione and protein thiols (6). The covalent binding of NAPQI to cell proteins is considered the initial step in a chain eventually leading to cell necrosis (6). It has been established that a hepatotoxic dose of paracetamol depletes the endogenous glutathione level to below a threshold value (<20% of control), therefore permitting interaction of NAPQI with cell macromolecule (7).

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METHODS

Method of preparation of neem leaf extract (NLE): One kg of freshly collected, shade dried, powdered leaves of A. indica were ground in 4 litres of distilled water and allowed to soak overnight. The suspension was centrifuged at 5000 rpm for 20 min and filtered through a Whatman No. 1 filter paper. The supernatant fluid was allowed to evaporate in glass petri dishes under tube light to provide heat and to prevent dampness so that no organism growth occurs. When completely dry, the powder was collected by scraping and was stored. Stock solution of aqueous extract was prepared by dissolving 500 mg of the extract in 5 ml of distilled water (8).

Animal experiments: Albino rats of Wistar strain (HAU Hisar, 180–200 g) were housed in cages, with free access to standard diet and water. Diet was withdrawn 6 hours before paracetamol (PCM) administration but water was not restricted.

PCM suspension was prepared with 0.2% gum tragacanth in distilled water. Animals were divided into 3 groups of 10 each. Suspension and the leaf extract were given by an intragastric tube. The dose of 500 mg/kg of NLE was selected on the basis of pilot study.

Group I – Served as control (which received 0.2% gum tragacanth suspension in distilled water).

Group II – Rats received PCM (2 g/kg single dose).

Group III – NLE (500 mg/kg) was given orally, once daily for nine days, beginning 7 days prior to PCM and continued for 2 more days. On 8th day, PCM was also given in addition to NLE.

After 48 hours of PCM administration, the blood was withdrawn directly from the heart and serum was separated by centrifugation for biochemical studies. Whole livers were removed after sacrificing the animal by decapitation and preserved in 10% formal saline. By standard technique serial sections (5 micron) were cut and stained with haematoxylin and eosin. Aspartate aminotransferase (AST; EC 2.6.1.1) alanine aminotransferase (ALT; EC 2.6.1.2) levels in different groups were assayed in serum (9). Gamma glutamyltranspeptidase (γ-GT; EC 2.3.2.2) activity in serum was also determined (10).

RESULTS

Gross examination of rat liver from control group showed normal appearance, red colour, smooth and regular under surface without any evidence of haemorrhage and necrosis. PCM treated liver showed multiple areas of necrosis and massive haemorrhagic patches. Most of the livers were covered with white slough and there were multiple white patches indicating necrotic areas. Liver weights (%body weight) were decreased in PCM treated group as compared to control. Livers from NLE pre-treated group were almost normal in appearance regarding colour and under surfaces and organ weight.

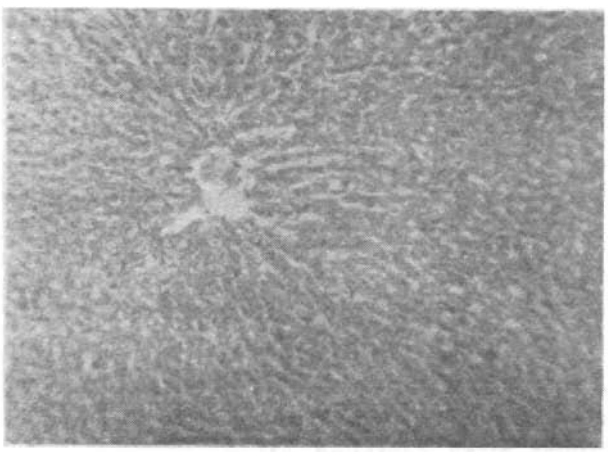

Fig. 1 : Liver section from normal rat showing portal triad, normal arrangement of hepatocytes with nuclei (low power 10x10).

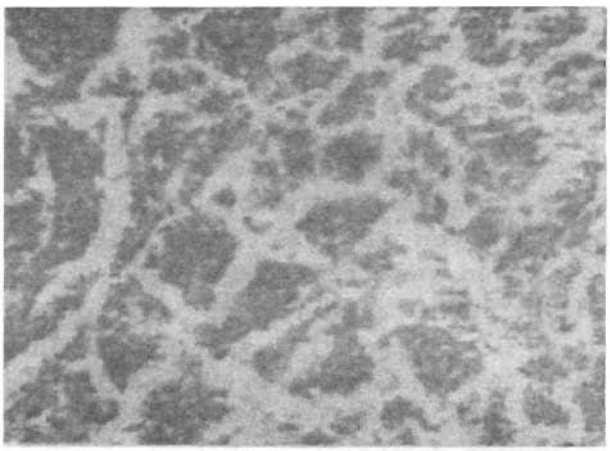

Fig. 2 : Liver section from PCM treated rat showing intense centrilobular necrosis, cloudy swelling, congestion of sinusoids with small lipid globules and gross hydropic vacuolation.

Histology of liver from control group showed portal triad, rows of hepatocytes or normal arrangements of hepatocytes with nuclei (Fig. 1), while PCM treated liver sections showed confluent centrilobular necrosis, cloudy swelling, pyknotic nuclei, loss of ribosomes, cytoplasmic matrix swelling and eosinophilic cytoplasm. There were small lipid glubules in surviving hepatocytes, congestion of sinusoids and gross hydropic vacuolation was prominent (Fig. 2). Histology of liver sections from NLE + PCM treated rats revealed few areas of congestion with mild fatty changes (Fig. 3).

TABLE I : Effect of NLE (500 mg/kg, p.o.) on serum AST, ALT and γ-GT levels in rats with PCM induced liver damage.

Groups	*Liver weight (g) (%body weight)*	*AST IU/L*	*ALT IU/L*	*γ-GT U/mI*
Group I (Control)	4.94±0.08	19.85±0.41	17.65±0.21	35.20±0.57
Group II (PCM)	4.16±0.10#	73.40±2.36#	74.70±1.12#	70.90±0.87#
Group III (NLE + PCM)	4.64±0.09**	24.65±0.33*	25.70±0.31*	41.35±0.22*

Values are Mean ± S.E. of 10 animals in each group.
#P<0.01 when compared with control.
*P<0.01, **P>0.05 when compared with PCM treated group.
(unpaired 't' test)

NLE-Neem Leaf Extract; AST-Aspartate aminotransferase;
ALT-Alanine aminotransferase; γ-GT-Gamma glutamyltranspeptidase;
PCM-Paracetamol.

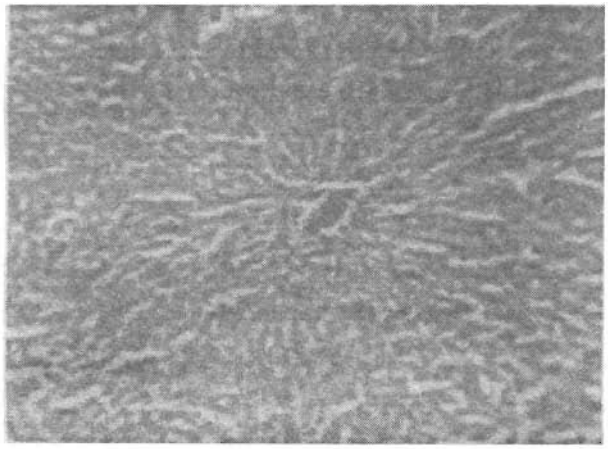

Fig. 3 : Liver section from NLE + PCM treated rat showing mild fatty changes with congestion.

Administration of PCM to rats produced a significant ($P<0.01$) elevation of serum AST, ALT and γ-GT levels as compared to control. There was a significant reduction in serum AST, ALT and γ-GT levels in rats treated with NLE + PCM as compared to animals treated with PCM alone, although the serum transaminase levels still remained higher in this group as compared to control (Table I).

DISCUSSION

Present study revealed marked protective effect of neem leaf extract (NLE) in PCM induced liver damage in rats.

Results of histological studies showed a marked reduction in the congestion of sinusoids and cloudy swelling in liver sections of rats pretreated with NLE as compared to rats treated with PCM alone. This indicates that NLE possesses some anti-inflammatory properties which may contribute to its hepatoprotective effects since hydropic swelling of mitochondria, endoplasmic reticulum and sinusoidal congestion are prominent features of PCM hepatotoxicity.

The highly significant ($P<0.01$) reduction in the levels of serum AST, ALT and γ-GT observed in the study in rats simultaneously treated with NLE and PCM as compared to PCM treated alone, also indicates that NLE affects important biochemical reactions which may be beneficial in reducing hepatic damage.

The hypolipidaemic effect of NLE may be beneficial in reducing PCM induced hepatotoxicity, since fatty changes have been reported in PCM induced hepatic damage. NLE has been reported to decrease serum lipids by others (2). We have also observed a fall in serum lipids in another series of experiments (unpublished observations).

The anti-lipoperoxidative property of NLE may also be contributing towards its hepatoprotective property, since it has been shown to be rich in flavonoid contents (11) and flavonoids are well known antioxidants (12).

It may be concluded from the observations in our study that A. indica leaf extract may have a protective effect against PCM induced hepatotoxicity in rats. However, further studies using some more models of experimental hepatic damage are required to elucidate exact molecular and biochemical mechanisms involved and to establish its therapeutic role as a hepatoprotective agent.

ACKNOWLEDGEMENT

The authors acknowledge the contribution by Dr. S. K. Mathur, Assoc. Professor, Department of Pathology, for the invaluable help in histopathological examination.

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