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Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: Part II

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Abstract

Hepatoprotective activity of *Azadirachta indica* leaf extract against paracetamol induced hepatic damage in rats has already been reported. In the present investigation effects of *Azadirachta indica* leaf extract on blood and liver glutathione, $Na^{+}K^{+}$-ATPase activity and thiobarbutiric acid reactive substances against paracetamol induced hepatic damage in rats have been studied with a view to elucidate possible mechanism behind its hepatoprotective action. It was interesting to observe that *Azadirachta indica* leaf extract has reversal effects on the levels of above mentioned parameters in paracetamol hepatotoxicity. Possible mechanism behind the results are discussed.

1. Introduction

In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cells. There are however, a number of drugs employed in traditional system of medicine for liver affections. Many formulations containing herbal extracts are sold in the Indian market for liver disorders. But management of liver disorders by a simple and precise herbal drug is still an intriguing problem.

Azadirachta indica (Meliaceae; Neem) has great reputation in Ayurvedic medicine for treatment of liver disorders. No scientific and methodical investigations have so far been reported in literature regarding its action on liver. Studies conducted in our laboratory reveals that water soluble portion of alcoholic extract of leaves of *Azadirachta indica* possesses significant antihyperglycemic, antiserotonin, antiinflammatory, hypotensive, hypolipidemic and antifertility activity (Chattopadhyay et al., 1986, 1987a,b; Chattopadhyay, 1993, 1995, 1997) and also have significant hepatoprotective activity against paracetamol induced hepatic damage in rats (Chattopadhyay et al., 1992). The present investigation has therefore been designed to study the effect of *Azadirachta indica* leaf extract on other biochemical parameters against paracetamol induced hepatic damage in rats with a view to elucidate possible mechanism behind its hepatoprotective action.

2. Materials and methods

2.1. Collection of plant material

Fresh matured leaves of *Azadirachta indica* were collected from our Institute's (Indian Statistical Institute, Kolkata, India) garden and were identified by a pharmacognosy expert. At the time of collection standard herbarium record sheets were completed with the name of the collector, collection number, specimen number, locality and local name.

2.2. Extraction of plant material

Air-dried powder (1 kg) of *Azadirachta indica* leaves were extracted by percolation at room temperature with 70% EtOH. Leaf extract of *Azadirachta indica* was concentrated under reduced pressure (bath temperature 50 °C) and dried in a vacuum desicator. The residue was dissolved in distilled water and filtered. The filtrate was evaporated to dryness. The dried mass (yield = 50.2 g) was diluted with normal saline and used in experiments.

2.3. Animals

Male albino rats of Wistar strain (100–150 g; 4–6 weeks old) were maintained under controlled conditions of light

(12 h/24 h) and temperature (23 ± 1 °C). Food pellets (Hindustan Lever Ltd., Mumbai, India) and tap water were provided ad libitum. For experimental purposes animals were kept fasting overnight but were allowed free access to water.

2.4. Paracetamol hepatotoxicity

The leaf extract/paracetamol/saline were given with the help of feeding cannula. Three groups (Group I, Group II and Group III) of rats, six rats in each group were taken. The leaf extract at a fixed dose (500 mg/kg, p.o.) which was found to be the working dose in our previous experiments, was fed daily for 7 days to one group (Group III) of rats and paracetamol (2 g/kg, p.o.) was administered on 5th day after 5th administration of the extract. The normal control group (Group I) and the paracetamol treated group (Group II) received normal saline in place of leaf extract. After 48 h of paracetamol feeding rats were sacrificed by cervical dislocation for estimation of blood glutathione, reduced liver glutathione, liver Na^+K^+-ATPase activity and liver thiobarbutiric acid reactive substances using standard methods.

2.5. Assay of liver glutathione and blood glutathione

Freshly collected livers were washed with 0.9% NaCl, weighed and homogenates were made in a ratio of 1 g of wet tissue to 9 ml of 1.15% KCl by using motor driven Teflon-pestle. Reduced glutathione (GSH) was estimated using DTNB (Sedlak and Lindsay, 1968). The blood glutathione was estimated by the method of Beutler (Beutler et al., 1963). The absorbance was read at 412 nm.

2.6. Liver Na^+K^+-ATPase activity

To measure the liver Na^+K^+-ATPase activity the liver was dissected out quickly, rinsed with cold phosphate buffer, liver plasma membranes were isolated and subjected for the estimation of Na^+K^+-ATPase activity (Corcoram et al., 1987).

2.7. Thiobarbutiric acid reactive substances (TBARS)

The concentration of TBARS was measured in liver using the method of Ohkawa et al. (1979). The concentration of TBARS was expressed as *n* moles of malondialdehyde per mg of protein using 1,1,3,3,-tetra-ethoxypropane as the standard.

2.8. Statistical analysis

The data were expressed as mean ± S.E.M. and statistically assessed by one way analysis of variance (ANOVA). The difference between drug treated animals and controls was evaluated by Student's *t*-test (Scheff'e, 1953).

2.9. Results

The glutathione level in liver homogenate and in blood, liver Na^+K^+-ATPase and liver thiobarbutiric acid reactive substances are given in Table 1. The concentration of GSH in animals treated with paracetamol was significantly reduced in homogenate of liver and so was the level of glutathione in blood and Na^+K^+-ATPase level as compared with saline control animals. While thiobarbutiric acid reactive substances of paracetamol treated animals was significantly higher than the saline treated control animals. Administration of *Azadirachta indica* leaf extract increased the concentration of GSH in liver and glutathione in blood and liver Na^+K^+-ATPase activity significantly when compared to its paracetamol treated control group. On the other hand, the increased level of liver thiobarbutiric acid reactive substances of paracetamol treated animals was significantly reduced in group of animals receiving both *Azadirachta indica* leaf extract and paracetamol.

2.10. Discussion

Liver is the organ highly affected primarily by toxic agents and so the study of above mentioned parameters have been found to be of great importance in the assessment of liver damage. From the foregoing findings it can be speculated that (i) the observed lowering effect of GSH,

Table 1
Effect of *Azadirachta indica* leaf extract on glutathione (blood and liver), liver Na^+K^+-ATPase, and liver thiobarbutiric acid reactive substances in rats subjected to paracetamol toxicity

Group	Blood glutathione (mg%)	Liver glutathione (umoles/g liver)	(Na^+K^+-ATPase (U/mg protein)	Thiobarbutiric acid reactive substances (*n* mol of MDA/g of wet tissue/h)
Group I saline control (2 ml/kg, p.o.)	1.27 ± 0.04	10.04 ± 0.21	9.98 ± 0.64	364.7 ± 9.8
Group II paracetamol treated control	0.57[a] ± 0.03	8.12 ± 0.36[b]	6.90[b] ± 0.27	442.4[a] ± 11.5
Group III paracetamol + *Azadirachta indica* leaf extract	1.00[c] ± 0.03	9.47[d] ± 0.24	9.42[c] ± 0.33	371.6[c] ± 8.28

Results are mean of six observations ± S.E.M.
[a] $P < 0.001$ when compared with normal control (Group I).
[b] $P < 0.01$ when compared with normal control (Group I).
[c] $P < 0.001$ when compared with paracetamol treated control (Group II).
[d] $P < 0.01$ when compared with paracetamol treated control (Group II).

blood glutathione, liver Na^+K^+-ATPase activity and increasing effect of liver thiobarbutiric acid reactive substance level in rats treated with paracetamol alone were due to hepatocellular damage and (ii) *Azadirachta indica* leaf extract afforded protection from such paracetamol induced liver damage. Possible mechanism that may be responsible for the protection of paracetamol induced liver damage by *Azadirachta indica* leaf extract include the following (i) *Azadirachta indica* leaf extract by itself could act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. Thus, by trapping oxygen related free radicals *Azadirachta indica* leaf extract could hinder their interaction with polyunsaturated fatty acids and would abolish the enhancement of lipid peroxidative processes leading to MDA formation. (ii) *Azadirachta indica* leaf extract pretreatment exhibited a novel effect on the glutathione status of the blood and liver cells. *Azadirachta indica* leaf extract significantly increases the hepatic content of GSH and blood glutathione. These results suggests that a higher content of glutathione in blood and liver would afford the tissue a better protection against an oxidative stress, thus contributing to the abolishment of paracetamol induced hepatotoxicity. (iii) The activities of Na^+K^+-ATPase is decreased in paracetamol induced animals. *Azadirachta indica*leaf extract prevented this effect of paracetamol. Therefore, *Azadirachta indica* leaf extract may be useful agent for the normalization of paracetamol induced impaired membrane function. Our previous findings (Chattopadhyay et al., 1992) reveals that the values of serum enzymes (AST, ALT, ALP) were much elevated in animals receiving paracetamol alone than in those receiving a combination of paracetamol and *Azadirachta indica* leaf extract in comparison with normal control animals indicating that the degree of hepatic cell damage was of lesser magnitude in extract treated group. These findings were confirmed by histological observations of liver (Chattopadhyay et al., 1992). Chemical analysis reveals that the leaf extract contains following six compounds (Chattopadhyay, 1999).

(i) Quercetin-3-*O*-β-D-glucoside;
(ii) Myricetin-3-*O*-rutinoside;
(iii) Quercetin-3-*O*-rutinoside;
(iv) Kaempferol-3-*O*-rutinoside;
(v) Kaempferol-3-*O*-β-D-glucoside;
(vi) Quercetin-3-*O*-α-L-rhamnoside.

It is well documented that quercetin, routine, Vitamin E are strong antioxidants. It is presumed that quercetin and rutin compounds of *Azadirachta indica* leaf extract may be responsible for hepatoprotective activity.

Thus, from the foregoing findings, it was observed that *Azadirachta indica* leaf extract is a promising hepatoprotective agent and this hepatoprotective activity of *Azadirachta indica* leaf extract may be due to its antioxidant and normalization of impaired membrane function activity. Further studies both on the extract and/or its chemical constituents are needed to pinpoint the findings. This report may serve as a footstep on this aspect.

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