

# Hepatoprotective Activity of Methanolic and Aqueous Extracts of *Azadirachta Indica* leaves

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**ABSTRACT:** *Azadirachta indica* had ample therapeutic action since many years. Hepatoprotective effect of methanolic and aqueous extracts of *azadirachta indica* leaves was evaluated using Male Wistar rats. Result indicate that the plant good potential to act as hepatoprotective agent.

**Keywords:** *Azadirachta indica*, Carbon tetrachloride; Hepatoprotective activity; Antioxidant activity; Silymarin.

## INTRODUCTION

*Azadirachta indica* is well known for its wide range of therapeutic uses. Different parts of the neem tree have been used as traditional ayurvedic medicine in India. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter.<sup>1</sup> Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident. Neem oil finds use to control various skin infections. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis . *Azadirachta indica* also posses immunostimulant activity, hypoglycaemic activity, antiulcer effect Antifertility effect, Antimalarial activity, Antifungal activity, Antibacterial activity, Antiviral activity, Anticancer activity.<sup>2-4</sup> In present study, in-vitro hepato protective effect of methanolic and aqueous extract of leaves of *A. indicais evaluated*.

## MATERIALS AND METHODS

### Plant Material

The leaves of *Azadirachta indica* were collected from botanical garden of B.K modi Govt Pharmacy College and was identified by institutional committee. Leaves were shade dried and extracted with methanol. One

part of powdered leaves was decocted in boiling water and the other part was macerated for 7 days in water with occasional stirring. The decoction and maceration were filtered and vacuum dried.<sup>5</sup>

### Animal

Male Wistar rats (180–200 g) were obtained from Cadila Laboratory Animal Center, Ahmedabad. They were housed in animal care facility under controlled environmental conditions (room temperature 25±1 °C with 12-h light:12-h dark cycle, relative humidity of approximately 60%) with standard rat pellets and tap water.

### Hepatoprotective activity

In vitro studies involved isolation of hepatocytes and examination of the effect of toxicants along with the test samples. The rat hepatocytes were isolated according to Seglen (1975) with slight modifications (Visen et al., 1991a) by recirculating enzymatic perfusion technique (in situ). The hepatocytes thus isolated were kept in the medium in petridishes for 15 min at 37 °C. The petridishes were divided in five groups of three petridishes each. Group A was kept as normal, group B was given CCl<sub>4</sub> treatment (10 µl), group C CCl<sub>4</sub> + aqueous extract (10, 50, and 100 µg/ml) and group D CCl<sub>4</sub> + methanolic extract (10, 50 and µg/ml).group E CCl<sub>4</sub> + Silymarin(15µg/ml,

30µg/ml, 90µg/ml). The viability of the cells was determined by trypan blue exclusion method (Visen et al., 1991b).<sup>6-10</sup>

#### Cell viability

The viability of cells to exclude trypan blue was determined by incubating the cell suspension (0.1ml) with 0.4% trypan blue (0.9ml). Viability of the isolated cells was determined by Trypan blue exclusion assay by counting the number of stained and unstained cells (viable cells). The concentration of the viable cells were adjusted to  $1 \times 10^6$  cells per ml.<sup>10</sup>

#### Antioxidant activity

Antioxidant activity was determined by measuring the glutamyl pyruvate transaminase (GPT), reduced glutathione (GSH), alkaline phosphate level (ALP), asparatate transminase activity (AST) and enzyme activity. The enzyme assay was carried out by Reagent Kits maintained by Miles India Ltd. and the procedures were essentially those described in the literature available with kits. Estimations were made on Auto-analyser, Reitman and Frankel method (1957) was used for determining the enzyme activity in the supernatant of various groups.<sup>12</sup>

#### Stastical Analysis

Results are presented as Mean  $\pm$ SEM. and percentage degree of reversal against hepatotoxin by test. Total variation present in a set of data was analyzed through one-way analysis of variance (ANOVA). The

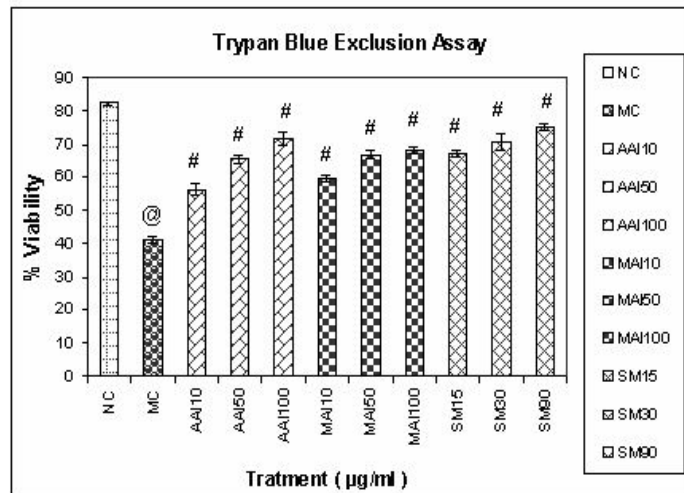
estimated *F*-ratio has been tabulated along with the critical value of *F*-ratio. Difference among means has been analyzed by applying Dunnet's '*t*' test at 99.9% ( $P < 0.001$ ) confidence level<sup>13,14</sup>

#### RESULT AND DISCUSSION

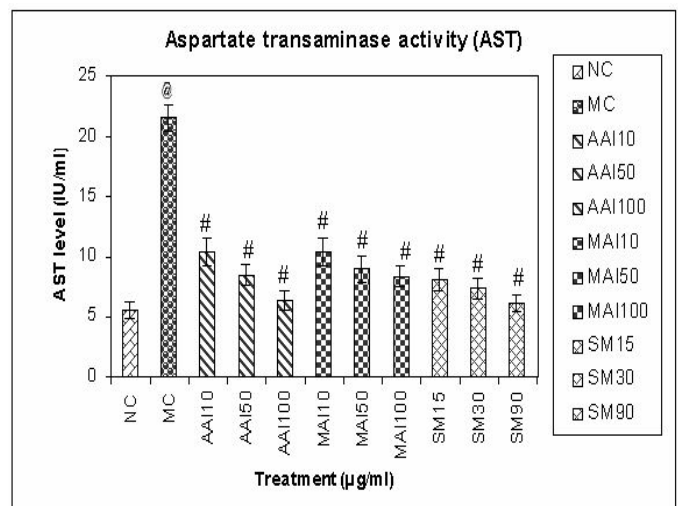
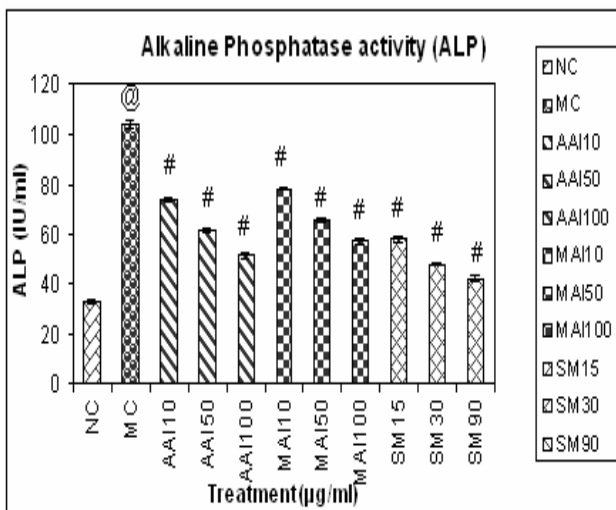
The hepatotoxicity induced by CCl<sub>4</sub> is due to its metabolite CCl<sub>3</sub> •, a free radical that binds to lipoprotein and leads to peroxidation of lipids of endoplasmic reticulum.<sup>15</sup> The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. Although enzyme levels and barbiturate sleeping time are not a direct measure of hepatic injury, they show the status of the liver. The lowering of enzyme level is a definite indication of hepatoprotective action of the drug. Protection of hepatic damage caused by carbontetrachloride treatment was observed by recording GSH, GPT, ALP and AST levels in treated, toxin control and normal groups because serum transaminases, serum alkaline phosphatase and serum bilirubin have been reported to be sensitive indicators of liver injury.<sup>16</sup> The disturbance in the transport function of the hepatocytes as a result of hepatic injury, causes the leakage of enzymes from cells due to altered permeability of membrane.<sup>17</sup> This results in decreased levels of GOT, GPT and alkaline phosphatase in the hepatic cells and a raised level in serum.

**Table 1: Antihepatotoxic activity of *Azadirchata indica* cultured rat hepatocytes @ Significant different from normal group at ( $P < 0.05$ ), # Significant different from control group at ( $P < 0.05$ ). Mean  $\pm$ SEM of six observations.**

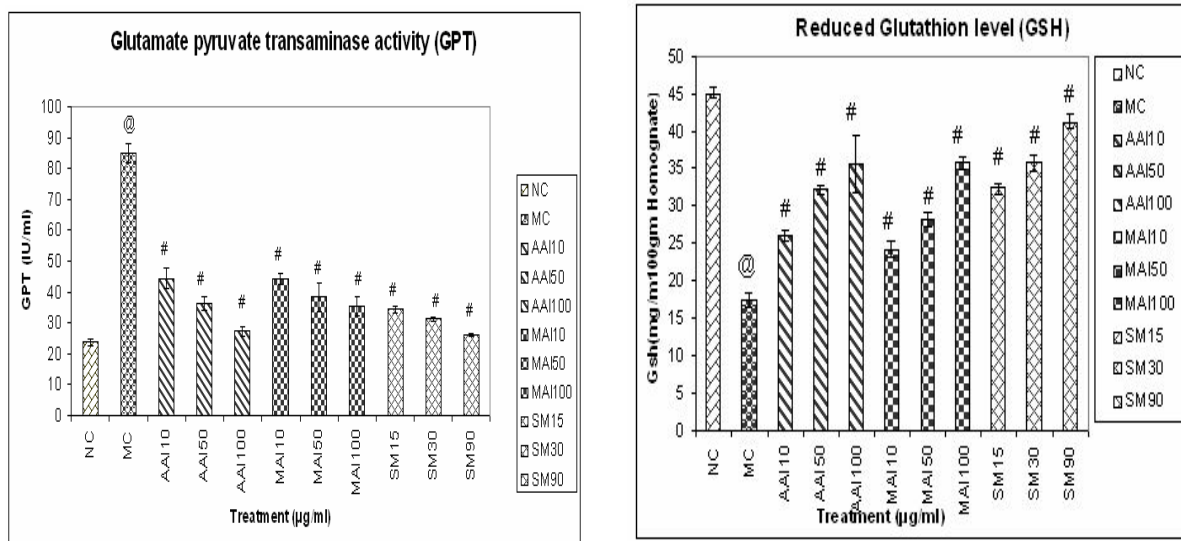
Group (n-6)	GPT(IU/ml)	ALP(IU/ml)	GSH(IU/ml)	AST(IU/ml)
NC	23.68 $\pm$ 1.4	32.89 $\pm$ 0.78	45.05 $\pm$ 0.68	5.6 $\pm$ 0.7
MC	85.26 $\pm$ 3.4@	104.15 $\pm$ 1.43@	1742 $\pm$ 0.94@	21.26 $\pm$ 2.18@
AAI10	44.33 $\pm$ 3.16#	73.99 $\pm$ 0.96#	25.98 $\pm$ 0.78#	10.45 $\pm$ 1.14#
AAI50	36.14 $\pm$ 2#	61.88 $\pm$ 0.68#	32.17 $\pm$ 0.6#	8.53 $\pm$ 0.83 #
AAI100	27.25 $\pm$ 1.57#	78.19 $\pm$ 1.00#	35.55 $\pm$ 3.78#	6.43 $\pm$ 0.83#
MAI10	44.28 $\pm$ 1.87#	78.19 $\pm$ 0.81#	24.08 $\pm$ 1.03#	10.45 $\pm$ 1.1#
MAI50	38.12 $\pm$ 4.69#	65.66 $\pm$ 1.08#	28.12 $\pm$ 0.98#	8.99 $\pm$ 1.1#
MAI100	35.39 $\pm$ 3.02#	57.23 $\pm$ 1.04#	35.69 $\pm$ 0.80#	8.35 $\pm$ 0.9#
SM15	34.33 $\pm$ 1.27#	57.95 $\pm$ 1.32#	32.27 $\pm$ 0.67#	8.1 $\pm$ 0.9#
SM30	31.07 $\pm$ 0.68#	48.24 $\pm$ 0.68#	35.68 $\pm$ 0.99#	7.33 $\pm$ 0.8#
SM90	26.08 $\pm$ 0.56#	42.11 $\pm$ 1.62#	41.19 $\pm$ 0.94#	6.15 $\pm$ 0.7#



**Fig 1: Effect of aqueous (AAI), Methanolic (MAI), extracts of *Azadirchata Indica* leaves and Silymarin on % viability of hepatocyte. Each bar represents Mean ±SEM of six observations. @ Significant different from normal group at (P< 0.05), # Significant different from control group at (P< 0.05).**



**Fig 2: Effect of aqueous (AAI), Methanolic (MAI), extracts of *Azadirchata Indica* leaves and Silymarin on alkaline phosphatase activity and aspartate transaminase activity of hepatocyte. Each bar represents Mean ±SEM of six observations. @ Significant different from normal group at (P< 0.05), # Significant different from control group at (P< 0.05).**



**Fig 3: Effect of aqueous (AAI), Methanolic (MAI), extracts of *Azadirchata Indica* leaves and Silymarin on glutamate pyruvate transaminase activity and glutathione level of hepatocyte. Each bar represents Mean ±SEM of six observations. @ Significant different from normal group at (P< 0.05), # Significant different from control group at (P< 0.05).**

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