

## ***Aegle marmelos* PROTECTS HEPATOCYTES FROM PARACETAMOL INDUCED HEPATOTOXICITY**

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### **ABSTRACT**

The present study was conducted to evaluate the hepatoprotective activity of hydroalcoholic extract of *Aegle marmelos* against paracetamol induced liver damage in rats. The methanolic extract of *Aegle marmelos* (500mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (3gm/kg). Silymarin (25mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5% CMC (Carboxy methyl cellulose) solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the methanolic extract of *Aegle marmelos* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

**KEYWORDS :** Paracetamol, Hepatoprotective and Hepatotoxicity, *Aegle Marmelos*, Paracetamol

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. *Aegle marmelos* leaves (Bael, family of Rutaceae) which is also called as Bilva in ancient Sanskrit, was used as herbal drug in the Indian System of medicine. The hepatoprotective effect of *Aegle marmelos* in alcohol-induced liver injury was evaluated rats using essential marker biochemical parameters. The results indicated that, the Bael leaves have excellent hepatoprotective effect. Similar findings were also reported by other workers<sup>1</sup>. It is found in the Indian Pharmacopoeia and is the prominent in at least 26 Ayurvedic formulas; whereas in Traditional Chinese Medicine (TCM). From ancient period medicinal plants play a vital role in the treatment of several diseases. In traditional systems of medicine many plants were used as cure for liver problems. Medicinal plants possess valuable bioactive compounds that protects human from various complications. *Aegle marmelos* is one such plant that was used quite often in traditional system of medicine. It belongs to the family

Rutaceae, and popularly known as “Bael tree” (Gamble, 1993 and Mathew, 1985). It is indigenous to India and found wild all over the SubHimalayan forests, in Central, and South India. The plant is reported to have multiple therapeutic properties such as antiinflammatory, antipyretic and analgesic (Arul and Dhananjyan, 2005), anti diabetic (Kamalakkannan et al., 2005 and Arumugam et al., 2008), antifungal (Rana et al., 1997). and antimicrobial, antibacterial and anti parasitic (Ulahannan et al., 2008), anti cancer (Gangadevi and Muthumary, 2008) and hepatoprotective (Singh and Rao, 2008) activity. Liver diseases are considered as fatal & life threatening. It creates a serious challenge to public health. Liver diseases are due to infection and/or exposure of liver to various toxic substances such as drugs or alcohol. Some times over dosage of drugs can also lead to liver damage. Now-a-day's due to inadequacy of liver protective agents, researchers and traditional medicine practioners concentrate in herbal based remedies for various liver disorders. Modern medicines have little to offer for alleviation of hepatic disorders. There was no safe hepatoprotective drug available for the treatment of liver disorders. Therefore, many folk remedies from plant source are used for the protection of hepatic damages starting from ancient period. Hence the present work was undertaken to scientifically prove the hepatoprotective nature of *Aegle marmelos* by an in-vivo study.

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## MATERIALS AND METHODS

### Plants and Animals

The plant samples were obtained from the campus of Banaras Hindu University, Varanasi (25.5°N 82.9°E). Albino rats of Charles Foster (CF-Alb) strain of either sex weighing between 180-250gm were procured from the Central Animal House Institute of Medical Sciences, Banaras Hindu University Varanasi. All the animals were kept in colony cages at an ambient temperature of 25±2°C with 45-55% relative humidity and 10:12 h light and dark cycle. Animals were kept on standard rodent diet and water *ad libitum*. All the experimental animals were acclimatized in the department for 3 days. Principles of laboratory animal care (NIH Publication No. 86-23 revised 1955) guidelines were followed after approval from the Institutional Ethics Committee.

### Preparation of Plant Extracts

The dried rhizome of *Aegle marmelos* and the leaves of *Aegle marmelos* were powdered with mechanical grinder and extracted with water and methanol (Me-OH) successively in *Soxhlet apparatus* (Borosil, Mumbai) for sixty hours. The water and methanol extracts after filtration were concentrated in *vacuo* under reduced pressure below 50°C to remove traces of solvent and stored in a desiccators until further use.

### Bioassays For Liver Marker Enzymes

The serum samples were subjected to assay for hepatic marker enzymes such as Aspartate transaminase (AST) Alanine transaminase (ALT) and Alkaline phosphatase (ALP). Chemicals and analyzing kits for analyzing different parameters of liver enzymes like AST, ALT, ALP, Bilirubin and Protein were procured from Avicon diagnostics (Varanasi). Activities of AST and ALT were assayed according to the 2-4 DNPH method (Kind and King, 1954). The lipid peroxidation (LPO) in the liver and serum were determined by the method and was expressed as n-mol of (nmole/ml) MDA. Simply 200µl of sample were added 200µl of SDS (8.1%), 1.5 ml of acetic acid (20%), 1.5ml of TBA buffer (0.8%) and mixture made up to 4ml with DDW and then heated in water bath at 95°C for 60min. After cooling with tap water, 1ml of DDW and 5ml of n-butanol and pyridine were added and shaken vigorously. After centrifugation at 4000rpm for 10min, butanol layer

was taken out and its absorbance was measured at 532nm, where TEP was used as external standard. The (superoxide dismutase) SOD level in serum and liver tissue were measured by using modified (Kakkar et al. 1984). Assay mixture contained 1.2ml of sodium pyrophosphate, 0.1ml of PMS, 0.3ml NBT; 0.2ml of NADH and 0.2ml tissue homogenate and 1ml of distilled water. Reaction was started after addition of NADH and incubation at 37°C for 60 seconds, reaction was stopped by adding 1ml of glacial acetic acid and stirred vigorously and shaken with 4ml of n-butanol. The mixture was allowed to stand for 10min and centrifuged at 4000 rpm, for 20 minutes and butanol layer was taken out. Colour intensity of chromogen was measured against n-butanol. One unit of enzyme activity is defined as enzyme concentration required inhibiting the absorbance at 560nm of chromogen induction by 50% in 1 minute under assay conditions.

### Statistical Analysis

The results were expressed as mean S.D of six animals from each group. The statistical analysis of variance was carried out by one way analysis of variance (ANOVA) P values < 0.05 were considered significant.

## RESULTS AND DISCUSSION

*Aegle marmelos* is one of the important herbs comprising several medicinal properties. *A. paniculata* is belongs to the family Rutaceae. *Aegle marmelos* has been found to be an effective antibiotic, antiviral, anti-parasitic and immune system stimulant. Hepatoprotective activity of *A. paniculata* against paracetamol was studied. The study brings about the potential hepatoprotective activity of *Aegle marmelos*, and gives insight into its mechanism of action. PCM, carbon tetrachloride etc are known to cause hepatocellular damage and are commonly employed as experimental hepatotoxic agents. An obvious sign of hepatic injury is leakage of cellular enzymes into the plasma. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepato cellular damage. In the present study the MDA and SOD levels were estimated. Any oxidative damage to a cell induces lipid peroxidation of cell membrane lipids. In the

Table 1 : Hepatoprotective Effect of Methanolic Extract of *Aegle Marmelos*

Groups	MICE (N=6) (12 days treatment)	SGOT (IU/ml)	SGPT (IU/ml)	ALP (KA. U/ml)	MDA (nmole/ml)	SOD (mg/ml)	AST (IU/ml)	ALT (IU/ml)
Control	Normal subjects without treatments	36.08 ± 1.4	29.58 ± 1.7	28.64 ± 1.4	14.83 ± 1.3	2.22 ± 0.03	42.69 ± 1.68	40.33 ± 2.31
Treated Control	Paracetamol treatment 2mg/kg b.w.	53.4 ± 1.7	41.24 ± 1.6	35.16 ± 2.4	19.89 ± 1.4	1.37 ± 0.01	67.53 ± 1.71	62.73 ± 2.36
Group I	<i>A. marmelos</i> extract 250mg/kg b.w.	43.38 ± 1.8	33.99 ± 1.4	36.11 ± 1.3	18.00 ± 1.0	1.25 ± 0.02	55.34 ± 4.04	55.83 ± 2.99
Group II	<i>A. marmelos</i> extract 500g/kg b.w.	33.05 ± 1.4	25.27 ± 1.0	26.55 ± 1.8	14.78 ± 1.3	2.24 ± 0.06	46.09 ± 3.57	41.47 ± 1.91

present study, we estimated SOD instead of GSH alone, which shows that a toxic dose of paracetamol caused a significant ( $P < 0.001$ ) reduction in SOD. SOD is an antioxidant enzyme its functions are concerned with the removal of free radical species such as hydrogen peroxide superoxide radicals. In this study the decreased level of superoxide dismutase (SOD) has been observed in paracetamol treated albino rats, where as its level was significantly found to be increased in *Aegle marmelos* treated, paracetamol induced albino rats. SOD is closely related to the direct elimination of reactive oxygen species. Ethanolic extract of *Aegle marmelos* (500mg/kg bw) when given orally for 21 days showed hepatoprotective activity in carbon tetrachloride induced hepatic damage in mice. Table 1 showed a significant increase in the level of liver enzymes like SGOT, SGPT, ALP and bilirubin in paracetamol intoxicated animals when compared with that of the control group of mice. Paracetamol an induced animal treated with plant extract, were slowly recovered from hepatic injury and was evidenced by lower level of liver enzymes. These results were observed in *A. marmelos* silymarin treated group. There was no change in plant control group. Hepatic injury caused by paracetamol administration at a dose of 0.2ml/kg body weight showed significant increase in the lipid profile, viz. total cholesterol, triglycerides, LDL & VLDL levels in liver tissue. Whereas HDL level was decreased as compared to that of control group of mice ( $p < 0.05$ ). However, treatment with *A. marmelos* at a dose of 500mg/kg and a known hepatoprotective agent silymarin (100mg/kg) to CCl<sub>4</sub> induced group of mice showed significant reduction in liver cholesterol, triglyceride, VLDL and LDL. On the other hand HDL level was increased compared to Paracetamol treated group and also no change was observed in plant control.

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