AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Protective effects of *Aegel Marmelos* leaves extracts against Carbon Tetrachloride induced Hepatotoxicity in rats.

Hiral K Modi*[1], Vishnu M Patel[2], Shweta M Bhadani[3]

1.Research Scholar, JJT University, Jhunjhunu, Rajasthan – 333001, India

2.APMC College of Pharmaceutical Education & Research , Himatnagar, Gujarat, India

3.Saraswati Institute of Pharmaceutical Sciences, Chiloda, Gandhinagar –382355, India

ABSTRACT

To evaluate the heapatoprotective activity of various extracts *of Aegle Marmelos*, belonging to the family Rutaceae against Carbon Tetrachloride (CCL_4) induced hepatootxicity in wistar Female rats. Oxidative stress play important role in many diseases. Hence, Herbal drugs play crucial role in treatment of various diseases due to its antioxidant property. The toxicant was used to induce hepatotoxicity at dose of CCl_4 1.25 ml/kg of body weight as mixture of 1:1 with olive oil for 30 days. Experimental groups of were constructed: a vehicle control group received the respective vehicles; a CCl4 group received a repeated single oral dose of CCl4 at 1.25 ml/kg; and the CCl4 &AMPE, AMCL.AMAL, AMAQ received repeated oral dose of *Aegel Marmelos* extracts in 500 mg/kg, respectively. It was found that AMCL, AMAL & AMAQ, at a dose of 500 mg/kg body weight exhibited hepatoprotective effect by lowering the Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), alkaline phosphate and total bilirubin to a significant extent. The groups treated with various extract of *A.Marmelos & Silyamarine* shows significant ($P<0.001$) restoration of liver weight & liver volume nearer to normal control group. Since results of biochemical studies of blood samples of *Aegle Marmelos* Extracts treated rats showed significant decrease in the levels of serum enzyme activities, reflecting the liver injury caused by CCL4 indicating the protection of hepatic cells against CCL4 induced hepatocellular injury. The effects of Aegel Marmelos extracts were comparable with standard drug silymarin.

Key words: *Aegle Marmelos*; CCl4 (Carbon Tetrachloride); Heaptotoxicity; Oxidative Stress; Protective Effects.

*Corresponding Author Email: Starhir86@gmail.com

Received 09 October 2012, Accepted 20 october 2012

Please cite this article in press as: Modi HK *et al.*, Protective effects of *Aegel Marmelos* leaves extracts against Carbon Tetrachloride induced Hepatotoxicity in rats. American Journal of PharmTech Research 2012.

INTRODUCTION

Liver disease is considered to be a serious health problem, as the liver is an important organ for the detoxification and deposition of endogenous and exogenous substances. Oxidative stress play important role in many diseases including liver diseases. The production of free radicals can be controlled by antioxidant system in living system. The liver is a key organ regulating homeostasis within the body & involved in almost all the biochemical pathway related to metabolism of fats, carbohydrates, proteins, hormones, synthesis and storage of vitamins, formation of bile, manufacture of antibodies, detoxification of drugs and other toxins, excretion of bilirubin & heavy metals.[1]

Hepatotoxicity means chemical-driven liver damage and chemicals that cause liver injuries are called hepatotoxins. The liver plays a central role in transforming and clearing chemicals and hence it is susceptible to the toxicity from these agents. Such unexpected toxicities appear to be the consequence of the unique vascular, secretary, synthetic, and metabolic features of the liver. Hepatocytes are highly reliant on ATP for ureagenesis, gluconeogenesis, and fatty acid metabolism among many other metabolic processes. So on long term deprivation of oxygen it leads to hepatocellular necrosis. Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality[2]. Carbon tetrachloride (CCl4), a well-known model compound for producing chemical hepatic injury, requires biotransformation by hepatic microsomal cytochrome P450 (CYP) to produce its the hepatotoxic metabolites, trichloromethyl free radicals (CCl3/$CCL_3$00).[2]

Jaundice & hepatitis are two major disorders of liver that increase the risk for mortality. Currently treatment options for hepatotoxicity are very limited. Free radicals initiate the damaging process through covalent binding to cell macromolecules leading to lipid peroxidation, oxidation of DNA and protein cause liver damage. Free radicals are found to be responsible for the toxic effects of xenobiotics. Flavonoids are a family of antioxidants that protect the cell from oxidative stress. Flavonoids and phenolic compounds, which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory activity, anticarcinogenic activity etc. Flavonoids used for the prevention and cure of various diseases which is mainly associated with free radicals[3]

Aegle Marmelos is a fruit-bearing tree indigenous to dry forest on hills and plains of central and southern Asian countries & belongs to the family Rutaceae. It has many Indian names, depending on the geographical region or the language.[5] Different parts of *A. Marmelos* have been

investigated by several workers and found to contain coumarins, alkaloids, triterpenes, sterols and essential oils & Flavonoids.[6] The objective of the present study was to investigate the hepatoprotective activity of the various leaves extract of the leaves of *Aegle Marmelos* using ethanol induced hepatotoxic rats.

MATERIAL & METHODS

Collection of Plant Material

The fresh leaves of *Aegle Marmelos were* collected from Ayurvedic Botanical Garden, Gandhinagar. Leaves were identified and authenticated at Mehsana, Gujarat. Fresh leaves dried under shade. The coarsely powdered fresh leaves were stored in polythene bags at room temp until required.

Extract Preparation

Successive Soxhlet Extraction

The dried and coarsely powdered material of *Aegle Marmelos* (100g) was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform and methanol. After each extraction, the solvent was recovered using distillation assembly. In vacuum after evaporation of ethanol from the ethanolic extract, residues were obtained and were stored in desiccators. Fresh Juice was prepared by just mixing of fine powdered material of *Aegle Marmelos* with water to make juice.

Animals

Wistar rats weighing 150-250g procured from central animal facility of Institute. The animals were maintained in controlled temperature (24 ± 2^0C) as well as humidity (60-70%) in 12 –h light –dark cycles with standard diet and water will provide ad libitium. The care and the use of these animals were in accordance with the guidelines of the CPCSEA. An experimental protocol was approved by IAEC

Hepatoprotective Activity

The animals (wistar female rats) weighing between 200 to 250 gm were divided into main four groups, six animals in each group. Animals in Group 1 were treated with Vehicle (Distilled water & olive oil) only single dose P.O for 30 days served as Normal control Group. Group 2 animals were treated with CCL4 1.25 ml/kg as mixture of 1:1 with olive oil as single dose P.O for 30 days Served as Positive control Group. Others animals were pretreated single dose with vehicle containing Petroleum Ether extract (AMPE), Chloroform extract (AMCL), Alcoholic Extract (AMAL), Fresh juice (AMAQ) of *Aegle Marmelos* 500mg/kg P.O for 30 days &

Silymarin 100 mg/kg P.O for 30 days, 1 hour before CCL4 1.25 ml/kg as mixture of 1:1 with olive oil administration. At the termination day, animals were anaesthetized using anesthetic ether and blood collected from retro orbital puncture. The level of SGPT, SGOT, TB and ALP were estimated as per the standard procedures described by manufacturer using serum kit.

Statistical Analysis

The results were expressed as mean ± SEM, where n represents the number of rats. Statistical difference between two means determined by one-way ANOVA followed by Dunnett t-test by using statistical computer software Graph pad Prism 5.0. Only those mean values showing statistical difference $P<0.05$ was considered as statistically significant.

RESULTS & DISCUSSION

Liver injury induced by CCl4 is a common model for screening the hepatoprotective activity of drugs because this chemical is a potent hepatotoxins and a single exposure can rapidly lead to increase in liver enzymes ,severe centrizonal hepatic necrosis and steatosis[7] Its metabolites such as trichloromethyl radical (CCL3) & trichloromethyl peroxy radical (CCL3OO) are involved in pathogenesis of liver. These free radicals bind to cellular molecules (nucleic acid, protein, lipid), impairing crucial cellular processes such as lipid metabolism (lipid peroxidation), which attacks and destroys polyunsaturated fatty acids, in particular those associated with phospholipids. Hence, permeability of mitochondrial, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage[8]

Increase in serum AST and ALT levels by CCl_4 have been attributed to hepatic structural damage because these entities are normally localized to the cytoplasm and are released into the circulation after cellular damage has occurred.[9]

The anti oxidative and free-radical scavenging activities of many Substances have been assessed, and many substances that possess anti-hepatotoxic activity also show strong anti oxidative activity.[3] *A. Marmelos* exhibits a number of beneficial effects against various types of degenerative diseases in humans, largely because its major ingredients, phenolic compounds & flavonoids, have potent anti oxidative activity[5]. The present in vivo study has demonstrated the hepatoprotective potential of *A. Marmelos* against hepatic injury produced by carbon tetrachloride in rats.

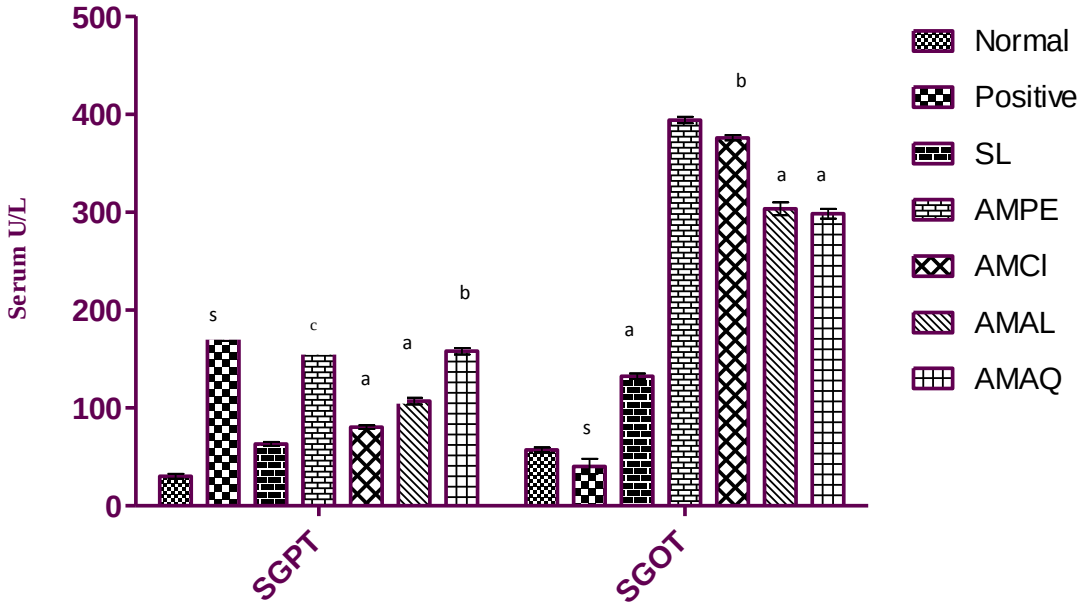

Figure 1: Effect of *A. Marmelos* Leaves extracts *Aegle Marmelos* Petroleum Ether (AMPE), *Aegle Marmelos* Chloroform Extract (AMCL), *Aegle Marmelos* Alcoholic Extract (AMAL), *Aegle Marmelos* Aqueous Extract (AMAQ) on SGPT & SGOT level in CCl4 intoxicated rats. Group I: Normal control, Group II: Toxin control CCL4, Group III: SL + hepatotoxins, Group IV: AMPE + CCL4, Group V: AMCL + CCL4, Group VI: AMAL+ CCL4, Group VII: AMAQ+ CCL4. Values are expressed in Mean ± S.E.M. Where n=5. $^{S}P<0.001$ designated as normal control versus disease control group. $^{c}p < 0.05$, $^{b}p < 0.01$, $^{a}p < 0.001$ as compared with toxin control group.

Amino transferases (SGPT & SGOT) contribute a group of enzymes that catalyze the interconversion of amino acids & α-keto acids by transfer of amino groups. These are liver specific enzymes which play sensible & reliable indices for necessary hepatotoxic as well as hepatoprotective or curative effects of various substances. Both of these SGPT & SGOT levels significantly (P< 0.001) increase in CCL4 treated groups due to toxic metabolite of CCl4 affects integrity of liver Kuffer cells when compare with normal control Group as shown in figure 1.

As shown in figure 1 Treatment with various extracts of *A. Marmelos* & Silyamarine significantly reduced the CCL4 induced Elevated levels of SGPT & SGOT. Upon Pretreatment *Aegle Marmelos* leaves extracts; (AMAL) in CCl4 intoxicated rats showed significant (P< 0.001) decrease in level of SGPT, SGOT & treatment with AMCL & AMAQ showed significant P<0.001 reduction in SGPT & SGOT respectively. Decreased levels of amino transferases indicate stabilization of plasma membrane protection of Hepatocytes against

damage caused by hepatotoxins. This is very simply accepted that serum levels of Transaminase returns to normal with the healing of parenchyma and regeneration of hepatocytes.

Furthermore, in present study in CCL4 intoxicated (Positive Control) group increase in the levels of serum Total bilirubin reflected the level hepatic damage and increase of Alkaline Phosphate level were the clear indications of cellular leakage and loss of functional integrity of cell membrane. Formation of ROS, oxidative stress and hepatocellular injury has been implicated to liver disease.[11]

In case of toxic liver, ALP (Alkaline Phosphate) level are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchyma or duct cells[3]. Pretreatment of *Aegle Marmelos* leaves extracts AMAQ, AMCL, AMAL in CCl4 intoxicated rats showed significant $P< 0.001$, $P<0.01$, $P<0.5$ decrease in level of ALP. Alkaline Phosphate is membrane found glycoprotein enzyme with high concentration in sinusoids & endothelium. The enzyme reaches to the liver mainly from the bone. It is excreted into the bile; therefore its elevation in serum occurs in hepatobiliary diseases. The results of present study indicate that the treated groups probably stabilize the hepatic plasma membrane from CCL4 induced damage which is shown in Figure 2.

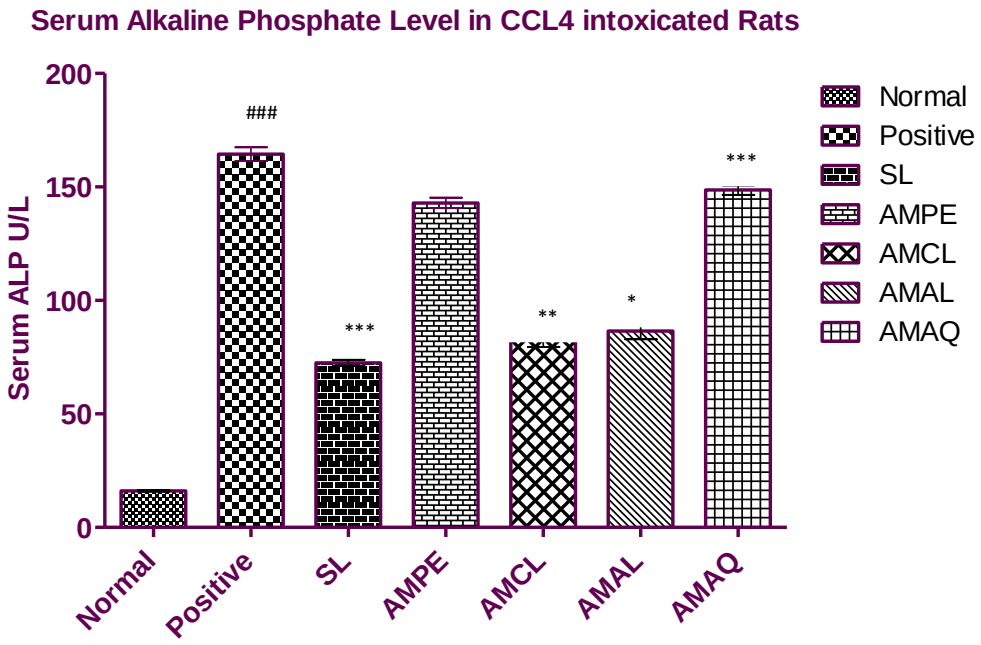

Figure 2: **Effect of A. Marmelos Leaves extracts *Aegle Marmelos* Petroleum Ether (AMPE), *Aegle Marmelos* Chloroform Extract (AMCL), *Aegle Marmelos*Alcoholic Extract (AMAL), *Aegle Marmelos* Aqueous Extract (AMAQ) Alkaline Phosphate level in CCl4 intoxicated rats.** Group I: Normal control, Group II: Toxin control CCL4, Group III: SL +

hepatotoxins, Group IV: AMPE + CCL4, Group V: AMCL + CCL4, Group VI: AMAL+ CCL4, Group VII: AMAQ+ CCL4. Values are expressed in Mean ± S.E.M. Where n=5. ### P<0.001 designated as normal control versus disease control group. *p < 0.05, **p < 0.01, ***p < 0.001 as compared with toxin control group.

In case of toxic liver, bilirubin level raised due to impaired hepatic uptake of unconjugated bilirubin. Such situation can occur in generalized liver injury, obstruction to biliary excretion into duodenum, in haemolysis & defects in hepatic uptake & conjugation of bilirubin pigment such Gibert's Disease. Pretreatment of *Aegle Marmelos* various leaves extracts (AMCL, AMAL, and AMAQ) in CCl4 treated rats showed significant (P< 0.001) decrease in level of Bilirubin. Pretreatment with AMPE in CCL4 intoxicated rats shows significant (P< 0.01) decrease in Total bilirubin when compare to disease control groups as shown in figure 3.

Reduction of alkaline phosphates levels with concurrent depletion of raised bilirubin levels suggests the stability of biliary function during injury with CCl4.

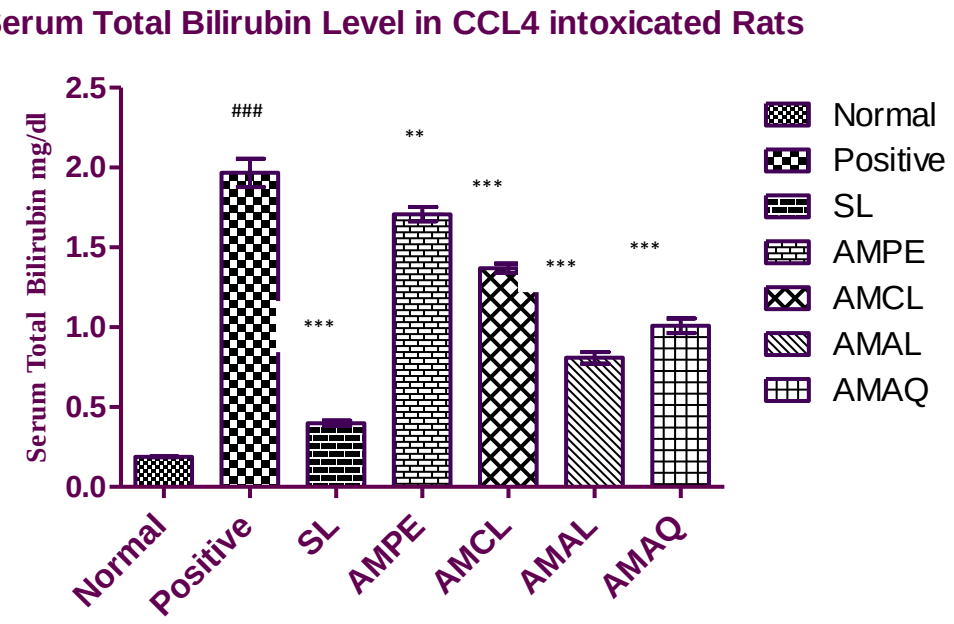

Figure 2: Effect of A.Marmelos Leaves extracts *Aegle Marmelos* Petroleum Ether (AMPE), *Aegle Marmelos* Chloroform Extract (AMCL), *Aegle Marmelos*Alcoholic Extract (AMAL), *Aegle Marmelos* Aqueous Extract (AMAQ) on Total Bilirubin level. Group I: Normal control, Group II: Toxin control CCL4, Group III: SL + hepatotoxins, Group IV: AMPE + CCL4, Group V: AMCL + CCL4, Group VI: AMAL+ CCL4, Group VII: AMAQ+ CCL4. Values are expressed in Mean ± S.E.M. Where n=5. ### P<0.001 designated as normal control

versus disease control group. *p < 0.05, **p < 0.01, ***p < 0.001 as compared with toxin control group.

CONCLUSION

The efficacy of hepatoprotective drugs is dependent on its capacity of either reducing the harmful effects or restoring normal hepatic physiology that has been distributed by hepatotoxins the Silyamarine group & the test groups decrease CCl4 induced elevated enzymes levels, indicating the protection of structural integrity of hepatic cell membrane or regeneration of damaged hepatic cells. It may be mentioned that the altered biochemical profiles due to CCl4 exposure is reversed towards normalization by *A. Marmelos* extracts but the effect was more pronounced with the AMAQ & AMCL leaves extract. The contents of the extract not only protect the integrity of plasma membrane but, at the same time increased the regenerative and reparative capacity of the liver. Beneficial effect of the extracts may be due to the presence of some phenolic components that have membrane stabilizing effects. These results suggests that the compound present in the plant extract efficiently works on the liver to keep it normally functioning and minimizing cell membrane disturbances.

REFERENCE

1. Ajith TA, Hema U, Aswathy MS. Zingiber officinale Roscoe prevents acetaminophen induced acute hepatotoxicity by enhancing hepatic antioxidant status. Food Chem Toxicol 2007 Nov; 45(11):2267-72.
2. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D. Mechanisms of hepatotoxicity. Toxicol Sci 2002 Feb ;65(2):166-76.
3. Recknagel RO, Glende EA, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther 1989;43(1):139-54.
4. Miller AL. Antioxidant flavonoids: structure, function and clinical usage. Alt Med Rev 1996; 1:103-111.
5. Pattanayak P, Mohapatra P. Phytopharmacology of *Aegle Marmelos* (L.) CORREA. Pharmacognosy Reviews 2008;2(4): Supplement
6. Siddique NA, Mujeeb M, Ansari SH, Ahmad S. Development of quality standards of *Aegle Marmelos* l. Leaves. Journal of Phytology 2010; 2(2): 36–43.
7. Zimmerman HJ, Chemical hepatic injury & its detection in toxicology of liver. In:plaa G, heweitt W,editors.New York: Reven Press;1982.p-1-45

8. Recknagel RO. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci 1983 Aug 1; 33(5):401-8.
9. Hwang YP, Choi JH, Jeong HG. Protective effect of the Aralia continentalis root extract against carbon tetrachloride-induced hepatotoxicity in mice. Food Chem Toxicol. 2009 Jan; 47(1):75-81
10. Bautista AP. Free radicals, chemokines, and cell injury in HIV-1 and SIV infections and alcoholic hepatitis. Free Radic Biol Med 2001 Dec 15; 31(12):1527-32.
11. Sergent O, Pereira M, Belhomme C, Chevanne M, Huc L, Lagadic-Gossmann D. Role for membrane fluidity in ethanol-induced oxidative stress of primary rat hepatocytes. J Pharmacol Exp Ther 2005 Apr; 313(1):104-11.